*United States Court of Appeals*

*for the*

*District of Columbia Circuit*

# TRANSCRIPT OF RECORD

UNITED STATES
COURT OF APPEALS FOR THE
DISTRICT OF COLUMBIA

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# BRIEF FOR APPELLANT

In the

# United States Court of Appeals

for the District of Columbia

Appeal No. 8715 586

F. J. STOKES MACHINE COMPANY, *Appellant*

vs.

CONWAY P. COE, Commissioner of Patents, *Appellee*

*APPEAL FROM THE JUDGMENT OF THE DISTRICT COURT OF THE UNITED STATES FOR THE DISTRICT OF COLUMBIA*

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IN THE

# United States Court of Appeals

FOR THE DISTRICT OF COLUMBIA

## Appeal No. 8715

F. J. STOKES MACHINE COMPANY, *Appellant*

vs.

CONWAY P. COE, Commissioner of Patents, *Appellee*

# BRIEF FOR APPELLANT

## Jurisdictional Statement

The complaint (App. 1)* was filed by appellant in the District Court under Section 4915 R.S. (35 U.S.C., Sec. 63) against appellee, the Commissioner of Patents, for a decree that appellant is entitled, as assignee of Flosdorf, Stokes and Weston, to have a patent issued to it containing claims identified in the complaint, on application Serial No. 219,858, filed July 18, 1938.

The Court below (Justice O'Donoghue) entered a Final Judgment on February 7, 1944, dismissing the complaint

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* We understand that Appellant's Appendix contains all of the portions of the record which will be referred to by both Appellant and Appellee and therefore will be the only Appendix in the case.

(App. 16). No opinion was rendered by the Court, but Findings and Conclusions submitted by counsel for the Commissioner of Patents, were entered (App. 13-15).

This Court's jurisdiction on this appeal is founded on the act of February 9, 1893, chapter 74, 27 Stat. 435, as amended and as incorporated in the District of Columbia Code, 1940 edition, Title 17, Sec. 101.

## Statement of the Case

The only question now presented in this case is as to whether the process defined by claims 28 and 35,* set forth in the complaint, constituted patentable invention over the prior art. These claims are as follows (App. 6):

> "Claim 28. The method of treating and packaging biological material for preservation which comprises, filling at least one clinical dose into a receptacle to not more than 75% of its capacity, said receptacle being suitable as a final container adapted for protection, distribution and restoration for use of contents, dehydrating the biological material by sublimation through the action of (1) a solid, porous chemical desiccant heat-regenerable without fusion and (2) vacuum until dry, while maintaining the material frozen by the auto-refrigeration of the sublimation, and sealing said receptacle to produce a final package ready for storage or distribution."

> "Claim 35. The method of treating and packaging biological material for preservation which comprises filling at least one clinical dose into a receptacle to not more than 75% of its capacity, said receptacle being suitable as a final container adapted for protection, distribution and restoration for use of the contents thereof, dehydrating the biological material by sublimation through the action of (1) a solid, porous chemical desiccant heat-regenerable without fusion and

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* Claims, other than Claims 28, 35 and 39, set forth in the Complaint were dropped at the trial. Claim 39, directed to apparatus, will not be pressed before this Court.

> (2) vacuum until dry, and sealing said receptacle to produce a final package ready for storage or distribution."

The process so defined is an improvement in the art of preserving biological products, such as sera, vaccines, antitoxins, venoms, and the like. These products, as originally produced, are in the form of aqueous liquids, which may contain 90% water and 10% solids, mostly protein in nature. They are labile products, and deteriorate on storage.

This deterioration presented serious problems to the biological industry and the medical profession for many years, because (1) it involved substantial economic loss, through the necessity of distributing most such products with a limited dating*; (2) it represents a distinct hazard**; and (3) some products, such as guinea-pig complement and human serum or plasma, simply cannot be stored for any substantial period in the liquid state regardless of what precautions are taken (App. 21, 87).

The disadvantages inherent in the marketing and distribution of biologicals in the liquid state in which they are produced and the impossibility or impracticability of marketing others, e.g., plasma and guinea-pig complement, have been known for many years. In the past, various kinds of preservatives have been tried, and various methods of eliminating water have been tried, but no successful method of preserving such products was known until the development, in the early 30's, of a process which has now come to be known as desiccation from the frozen state (App. 87).

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* A product such as diphtheria antitoxin is prepared and distributed to the trade with each container bearing a date say six months later than the date of distribution. If the product is not used before that date, it is returned to the manufacturer and, in most instances, is discarded. With this practice, a large proportion of biologicals which have been prepared and distributed have eventually been thrown away (App. 20, 87).

** The rate of deterioration of such products is not necessarily uniform. While dosage is not critical with many biological products, with others it is dangerous to inject too much, but necessary to inject enough. The amount to be injected is determined by the potency of the product determined before packaging or at the time of packaging. If the limitations as to necessary dose and dangerous dose are relatively close, the latent hazards in the use of a product which has suffered some loss in potency on storage is obvious, particularly where the exact degree of loss of potency cannot be known. Potency tests of these materials cannot be made at the time or place of administration (App. 21, 112).

In this process, the material, which usually contains around 90% of water, is frozen, usually by immersing a container with the material in it in a very cold bath, although other methods of freezing have been and are used.

The frozen material is then subjected to a high vacuum, while it is maintained below the freezing point. By keeping the material cold, so that it cannot melt, and applying the high vacuum, the frozen water in it is sublimed, that is, it passes directly from the solid state to the vapor state. This operation is continued until over 99.9% of the water originally present is removed, leaving a dry product with a water content of 1% or less (App. 18-21).

This dry product* is a porous mass having about the volume of the original frozen material. Upon the addition of water it readily goes into solution, forming a liquid product which is the full equivalent of the original product (App. 87, 107, 112).

In this desiccated form, the materials are remarkably stable. They may be preserved for years, even while maintained at ordinary temperatures. Thus guinea-pig complement, one of the most unstable of all biologicals, which in its ordinary liquid form loses almost all potency in 24 hours at ordinary temperatures has maintained its activity for as much as 5 years in the desiccated form (App. 21-22).

While in broad outline, the process is simple, the practical application of it has presented a number of substantial difficulties which have been overcome to a very considerable extent in the past decade. It is necessary, while the material is undergoing the desiccation, that it be kept frozen or solid. If any of it reaches a temperature sufficiently high for it to melt or soften, with removal of water from the melted portion by evaporation of liquid water, the product produced is of no value, and must be discarded, because it will not redissolve readily and because it usually suffers denaturation. This fact imposes the requirement that the sublimation be carried out at a very low pressure. For practical operation, this pressure must be less than 1 mm. of mercury. One volume of water, when converted to vapor at this low pressure gives approximately 4½ million volumes of water vapor. This huge amount of water vapor must be disposed of somehow, otherwise the sublimation will cease and the desiccation will not proceed (App. 18-20).

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* The outstanding and best known product of this process is blood plasma. It is no exaggeration to say that the development of the process of desiccation from the frozen state made possible the blood plasma program which is playing such an important role in the present war (App. 87).

In the process as originally developed, the means of removal of this water vapor was a cold condenser. The apparatus used consisted of a vessel containing the material to be desiccated, or a number of containers attached to a manifold, a large condenser communicating with that vessel or manifold, and a vacuum pump communicating with the condenser. In operation, the vacuum pump removed the air from the system, creating the vacuum necessary for the sublimation of the ice to take place. Then as water vapor was evolved from the frozen material, it flowed to the condenser, where it was condensed to ice. By maintaining the condenser at a temperature well below that of the frozen material to be desiccated *e. g.,*—70°C., and by eliminating air from the system by the use of the vacuum pump, conditions in the apparatus were such that the vapor pressure of the ice in the frozen biological was greater than that of the ice adhering to the condenser, so that the ice in the biological would sublime, and the vapor so formed would flow to the condenser and there be reconverted to ice. It was considered necessary to condense the water vapor, and to have little if any of it pass to the vacuum pump, because the vacuum pumps used were oil-sealed pumps, and the passage of any substantial amount of water vapor to or through the vacuum pump would result in condensation of the water, with resulting contamination of the sealing oil and impairment of functioning of the vacuum pump.

The process as so developed is well described in the Reichel patent 2,066,302, the Elser patent 1,970,956 and the Flosdorf patent 2,163,996, the primary references on which the allowance of claims 28 and 35 has been refused in this case (Def. Ex. 1, App. 104-115, 126-136).

## The Process of the Appealed Claims

The claimed process is one in which the desiccation is carried out by sublimation from the frozen state, but in which, instead of condensing the evolved water vapor in a cold condenser between the vacuum pump and the frozen material, the water vapor is absorbed in a desiccant, specifically a form of anhydrous calcium sulfate known commercially as Drierite. This product, as a desiccant, was known prior to the invention under discussion here.

No tribunal which has been called upon to decide the question presented in this case has held that the process as claimed is described in any prior patent or publication, and no tribunal has held that the process lacks novelty. The position taken by the three tribunals which have considered the question is that the process lacks invention on the ground that it was obvious to substitute for the cold condenser of the Elser, Reichel and Flosdorf patents a known desiccant, described for example in the Moore et al. patent 2,083,732 and the Hammond patent 1,887,349 (Examiner's Statement, App. 137-149; Decisions of Board of Appeals, App. 150-155; Finding of Fact 8, App. 15).

The process has commercial significance. There are in use today over 100 installations of apparatus built by appellant, the use of which involves the process under discussion. It is being successfully applied to the preservation of a wide range of biological products, both for research purposes and for commercial purposes. It is of particular importance for small scale operations, or for operations in out of the way places (App. 33-34).

The position taken by the Examiner in rejecting the claims was that there was no invention in substituting a desiccant for the cold condenser of the Reichel, Elser and Flosdorf patents, the desiccant itself being a known product. In its original decision, the Board of Appeals advanced a somewhat different ground in affirming the Examiner, but on reconsideration, it changed its viewpoint and sustained the Examiner's rejection on the ground advanced by the Examiner. The Court below wrote no opinion, so that it is impossible to say upon what reasoning its decision dismissing the complaint was based. The Findings of Fact signed by the Court are in line with the position taken by the Examiner and the Board of Appeals.

In taking this position, all three of the tribunals refused to give significance to what we regard as the controlling factors in the case. They are (1) that those skilled in the art explicitly taught that desiccants could not be used in such a process because of the danger involved and (2) that those skilled in the art lacked the knowledge necessary to use desiccants in such a process or to be led to believe that they could be used.

As to the first point, we think, in view of the record, it can be assumed that Elser and Reichel, who were largely responsible for the original development of the sublimation process, are adequate representatives of those skilled in the art. In the patents of both the process is described as one in which the vapor from the frozen material is condensed by a cold condenser interposed in the line of flow between the frozen material and the vacuum pump. Both Elser and Reichel, in their patents, made some reference to desiccants, and that reference was an explicit instruction that desiccants should not be used in the process because of the danger involved.

Thus Elser, in his patent, states:

> "It may here be noted that this system neither depends upon nor contemplates chemical absorbents for performing the desiccation. Although such agents, as for example, phosphorus pentoxide have a powerful affinity for water, and a very low vapor pressure, nevertheless they (or perhaps, certain impurities thereof) are volatile to the extent of their vapor pressures, and may bring about changes in the serum such as to defeat the purpose of the invention. This has been demonstrated to be a fact" (App. 107, ll. 74-84).

Reichel, in his patent, states:

> "It will also be noted that the material is protected, during the removal of water therefrom, while in a solid frozen state, from contact with the atmosphere, and from contact with or use of any of the known dessicating materials" (App. 114, Col. 2, ll. 43-47).

The Flosdorf patent, which is one of the major references in the case, describes as a major advantage of the particular type of apparatus to which the patent relates the fact that it makes unnecessary even "the provision of an absorbent tube of dehydrating material such as $P_2O_5$ between the condenser and the pump" (App. 130, Col. 2, ll. 45-48). This shows that Flosdorf, prior to the making of the joint invention here involved, regarded it as worthwhile to develop apparatus with a cold condenser so efficient as to make unnecessary the small tube of $P_2O_5$ normally positioned just prior to a vacuum pump to prevent contamination of the sealing oil.

These objections of such men as Reichel and Elser to the use of desiccants in the system had a sound foundation, and were not based upon any whimsical notion or any attempt to belittle some suggestion by others. The two common desiccants known were sulfuric acid and $P_2O_5$. $P_2O_5$ is the anhydride of phosphoric acid. It is fine, white powder which makes a very good smoke screen and has a decided tendency to fly (App. 27, Pl. Ex. 4). Sulfuric acid is a liquid (App. 29). What Elser and Reichel were afraid of was that a small amount of such a material might pass over to the frozen material, with possible serious consequences.

As previously noted, the materials to which this process is primarily applied are biologicals, most of them used by injection. Such acids as phosphoric and sulfuric acid, in concentrated form,* denature these protein materials. Admixture of either of these acids with a biological, would undoubtedly result in denaturation. Such denaturation, as caused by the localized introduction of a small, even minute amount of either, would probably not cause any change in the appearance of the product, but would involve the formation in the biological of a small amount of protein decomposition products. Injection of a product containing such decomposition products, particularly if given in the course of a series of injections, might well result in what is known as anaphylactic shock, resulting in death or severe illness. The risk of shock in the injection of protein decomposition products, especially to persons sensitized by previous injections, is well known. The danger involved is real. It is important to note that the real danger is from contamination with a small, even minute, amount of a product such as $P_2O_5$ or sulfuric acid, because contamination by a large amount would presumably result in changes in appearance which would be noticeable to one looking at the product and permit discarding the contaminated product. The previous prohibitions against the use of desiccants were based upon this reasoning, because once the material is introduced into the container and frozen, it is not susceptible of further examination or test, so that if contamination by a small amount of $P_2O_5$ or sulfuric acid took place, there would be, and can be, no way of determining that fact (App. 27-28).

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* For use as desiccants, $P_2O_5$ is used in the form of its anhydride, sulfuric acid in the form of a solution of maximum concentration.

The significance of the contribution represented by the process under discussion has been recognized by independent workers in the art, as shown by the publication of Hill and Pfeiffer in the "Annals of Internal Medicine" for August, 1940 (Pl. Ex. 7, App. 87, *et seq.*). Note particularly the statement on page 204 (App. 90) as follows:

> "A very much cheaper process, the Cryochem, was described by Flosdorf and Mudd in 1938. They resorted to chemical absorption but with much greater success than earlier workers because of their choice of a much more suitable chemical, specially prepared anhydrous calcium sulphate, known as 'Drierite.' This process, although the most economical to date, still has serious drawbacks to be discussed later."

The Cryochem process, referred to here, is the process of the claims on appeal (App. 22).

## Statement of Points

Appellant contends that the essential error of the District Court is in its finding No. 8 and that because of such error, reversal of the decision of the District Court is required. Recognizing that the rule applied by this Court in appeals of this nature is the usual rule with respect to cases involving findings by a District Court or by an administrative tribunal, in this case both, we say that the decision of the District Court should be reversed because:

(1) There is no evidence in the record to support finding No. 8 of the District Court, in that there is nothing in

the record to sustain the proposition that it was obvious to use a desiccant such as Drierite in place of a cold condenser. *Levin* v. *Coe,* 132 F. (2d) 589, 76 App. D. C. 347; *Gasoline Products Co.* v. *Coe,* 87 F. (2d) 550, 66 App. D. C. 333.

(2) The evidence affirmatively establishes that finding No. 8 is plainly wrong and that invention was involved in the use of a desiccant instead of a cold condenser, in that it shows (a) those skilled in the art warned against the use of desiccants and (b) knowledge of the properties of such desiccants as Drierite in respect of behavior under the exacting conditions of the involved process was not available to those skilled in the art, who therefore had no basis for considering the substitution possible or desirable. *Levin* v. *Coe, Gasoline Products Co.* v. *Coe, supra.*

## Summary of Argument

(1) There is no evidence in the record to show that it was obvious to use a desiccant in place of the cold condenser. The record shows that it was old to use a cold condenser for condensing the huge volume of water vapor in operations involving desiccation from the frozen state, and permitting the sublimation to proceed. The record shows that desiccants were known for the purpose of removing water from air or the like, and that the specific desiccants which are used in the process of the involved application were known to have the capacity of absorbing water from moist gases. The record does not show that anyone has heretofore contemplated or suggested the use of a desiccant to absorb enormous volumes of water vapor under a relatively high vacuum, or that there is any previously known industrial system or procedure in which a cold condenser and a desiccant are recognized as equivalents.

(2) The record affirmatively establishes that invention was involved in that (a) it shows that those skilled in the

art explicitly warned against the use of desiccants and taught that the use of desiccants was dangerous and to be avoided, and (b) it shows those skilled in the art did not have the necessary technical knowledge to believe the substitution possible or advantageous. This is persuasive evidence that invention was involved.

## ARGUMENT

### (1) Finding 8, upon which the District Court's decision is predicated, is unsupported by the evidence.

Finding 8, the foundation of the decision from which appeal is taken, reads as follows (App. 15):

> "8. It was not invention to use a chamber containing a desiccating agent, such as disclosed in the patent to Hammond and in the patent to Moore et al., in lieu of the condenser of the Flosdorf patent or that of the Elser or Reichel patents."

This is an unsupported conclusion as to the ultimate fact which is controlling in this case. It is a finding that one skilled in the art, familiar with the cold condenser operation described in the Elser, Reichel and Flosdorf patent, would find it obvious to substitute for the cold condenser the use of a desiccant such as Drierite.

This finding is supported in the record only to the extent that the record includes patents which describe a cold condenser process and patents which describe such desiccants as Drierite, an admittedly old, known desiccant.

This finding is not supported by evidence that anyone prior to the present inventors knew that such desiccants as Drierite have the capacity for absorbing enormous quantities of water vapor very rapidly at very low pressures, a requisite for use in desiccation from the frozen state. This finding is not supported by evidence which shows that anyone prior to the present inventors taught that a desiccant could be used in such a process, or that anyone contemplated the use of a desiccant in such a process, or that anyone regarded cold condensers and desiccants as equivalents.

Whatever the standards may be as to the quantity or weight of evidence required to support findings of fact, the rule is clear that there must be some evidence to support them, and, in the absence of some evidence, such findings of fact will be held erroneous. Findings which fail to reflect such facts, established by the evidence, as the lack of knowledge of those skilled in the art of the properties of desiccants under the conditions of operation and the specific warnings of those skilled in the art against the use of desiccants are erroneous and require reversal. *Levin* v. *Coe,* 132 F. (2d) 589, 76 App. D. C. 347.

In this connection we wish to point out that to sustain a finding such as Finding 8 in the present case, and to hold that such findings are appropriately made by the District Court in the absence of supporting evidence other than the patents which are cited and the conclusion that the transposition of material from one patent to another is obvious, would in effect eliminate Section 4915 of the Revised Statutes as the vehicle for review of the administrative proceedings of the Patent Office in cases involving the question of invention, because in every case involving the question of invention where the Patent Office refuses to grant claims for lack of invention, there is cited one or more patents in support of the rejection. The question which is presented is always whether the claimed subject matter is obvious to those skilled in the art familiar with the cited patents. We submit to this Honorable Court that, in such circumstances, there must be evidence in the record which supports the conclusion that the change involved, or as in the present case, the substitution involved, is one which is obvious to those skilled in the art. To warrant an adverse conclusion on the question of invention, it must appear that the change involved was one which simply involves the substitution of superior for inferior materials or the substitution of one material or element for another in which advantage is taken of the known properties of the substituted material or element, as distinguished from taking advantage of unknown properties thereof, or in which there is substitution of one material or element for its known equivalent. There is no such evidence in the present case.

### (2) The record affirmatively establishes that the substitution of a desiccant such as Drierite for the previously used cold condenser involved invention.

Here, we submit that the case of *Gasoline Products Co.* v. *Coe,* 87 F. (2d) 550, 66 App. D. C. 333, is in point and persuasive authority for recognition of invention in the present case. See also *Levin* v. *Coe,* 132 F. (2d) 589, 76 App. D. C. 347.

In the *Gasoline Products* case, the question involved was whether invention was involved in the substitution of chrome steel coils in cracking stills in place of previously used coils of various metals, for the cracking of sulfur-bearing oils. Invention was found on the ground that the inventor had discovered that the cause of the corrosion encountered with previously used coils was sulfur or sulfur compounds and had overcome the corrosion problem by the use of chrome steel coils in the absence of knowledge of the prior art as to the property of chrome steel of resisting sulfur corrosion, and despite the fact that the resistance of chromium steel to corrosion generally was well known.

In that case, as in this, there was a finding, although it was included in the conclusions of law rather than in the findings of fact, that no invention would be involved in making cracking coils, previously made of various materials, out of chrome steel in view of certain patents cited showing chrome steel coils to be old.

The concurrent holdings of the Patent Office and District Court were reversed because of the failure of the District Court and the Patent Office to give adequate weight to the affirmative proof that invention was present, which consisted of proof that the prior art lacked knowledge of what caused the corrosion and lacked knowledge of the specific sulfur-resistance of chrome steel.

A similar situation was presented in *Levin* v. *Coe, supra,* and a similar conclusion reached.

In the instant case, there is a substitution of a desiccant such as Drierite for a cold condenser. The finding of fact upon which the decision below was based is that there would be no invention in this substitution. The affirmative proof in this case parallels that in the *Gasoline Products* case in that the unquestioned evidence is (1) that those most skilled in the art specifically warned against the use of desiccants, specifically $P_2O_5$ and sulfuric acid, because they apprehended, in their use, a hazard so substantial as to preclude their use, and (2) that those skilled in the art did not know that such desiccants as Drierite had the capacity of absorbing large volumes of water vapor rapidly at low pressures. There is no evidence that cold condensers and desiccants were regarded as equivalents. The evidence is that Hammond, inventor and seller of Drierite, was surprised to learn it would function in the high vacuum process (App. 30).

## CONCLUSION

**We submit that the judgment appealed from should be reversed to the extent required for the issuance of a judgment authorizing the grant to appellant of a patent containing claims 28 and 35.**

Respectfully submitted,

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UNITED STATES
COURT OF APPEALS FOR THE
DISTRICT OF COLUMBIA

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# APPENDIX TO APPELLANT'S BRIEF

In the

## United States Court of Appeals

for the District of Columbia

### Appeal No. 8715

F. J. STOKES MACHINE COMPANY, *Appellant*

vs.

CONWAY P. COE, Commissioner of Patents, *Appellee*

*APPEAL FROM THE JUDGMENT OF THE DISTRICT COURT OF THE UNITED STATES FOR THE DISTRICT OF COLUMBIA*

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IN THE

# District Court of the United States

FOR THE DISTRICT OF COLUMBIA

CIVIL ACTION No. 17,515.

F. J. STOKES MACHINE COMPANY, Adams Avenue,
Philadelphia, Pennsylvania,
Plaintiff,

*v.*

CONWAY P. COE, Commissioner of Patents,
Washington, D. C.,
Defendant.

## COMPLAINT FOR ISSUANCE OF UNITED STATES LETTERS PATENT

(Filed October 19, 1942.)

I. The jurisdiction of this Court depends upon the Patent Laws of the United States, this Complaint being filed under Section 4915, Revised Statutes (Comp. Stats. 9460; U. S. C. Title 35, Section 63), as amended by Act of March 2, 1927.

II. Plaintiff, F. J. Stokes Machine Company, a corporation of the State of Pennsylvania, is by assignment in writing the sole owner of the entire right, title and interest in and to an application for United States Letters Patent of Earl W. Flosdorf, Charles J. Westin and Francis Joseph Stokes, Jr., for improvements in Biological Apparatus, Containers and Methods, filed in the United States Patent Office on July 18, 1938, serial No. 219,858, and the improvements disclosed and claimed therein.

III. The Board of Appeals of the United States Patent Office has refused to allow eighteen claims of the said application serial No. 219,858, which claims are as follows:

[2] *Claim 1.* A package for a dry, very porous biological substance of superior stability in amounts suitable for clinical use, conveniently and easily restorable to original consistency by adding water and occupying approximately the same space in the package as the original liquid and adapted to contain the biological substance during filling, freezing, sealing, storing and use comprising, a sealed container of ordinary glass not having a particularly low coefficient of thermal expansion.

*Claim 2.* A package for a dry, very porous biological substance of superior stability in amounts suitable for clinical use, conveniently and easily restorable to original consistency by adding water and occupying approximately the same space in the package as the original liquid and adapted to contain the biological substance during filling, freezing, sealing, storing and use comprising, a container of ordinary glass not having a particularly low coefficient of thermal expansion, and a seal for said container.

*Claim 3.* A package for a dry, very porous biological substance of superior stability in amounts suitable for clinical use, conveniently and easily restorable to original consistency by adding water and occupying approximately the same space in the package as the original liquid and adapted to contain the biological substance during filling, freezing, sealing, storing and use comprising, a container of ordinary glass not having a particularly low coefficient of thermal expansion, and a seal for said container comprising a stopper of flexible material having a wall of reduced thickness for puncturing by a sharp instrument such as a syringe needle.

*Claim 4.* An apparatus providing means for treating biological material for preservation by dehydrating through the action of a regenerable chemical desiccant and vacuum and for regenerating the desiccant comprising, a desiccant chamber, a solid, porous, regenerable chemical desiccant within the chamber, a plurality of outlets communicating with said chamber and for use as container connectors, and vacuum-producing means communicating with said chamber for the removal of air.

*Claim 5.* An apparatus providing means for treating biological material for preservation by dehydrating through the action of a regenerable chemical desiccant and vacuum and for regenerating the desiccant comprising a desiccant chamber, a solid, porous, regenerable chemical desiccant within the chamber, a manifold connected with said chamber, a plurality of outlets on said manifold suitable for use as container connectors, and vacuum-producing means associated with the chamber for the removal of air.

[3] *Claim 13.* An apparatus providing means for treating biological material for preservation by dehydrating through the action of a regenerable chemical desiccant and vacuum and for regenerating the desiccant comprising a desiccant chamber, a solid, porous, regenerable chemical desiccant within the chamber, a plurality of outlets communicating directly with the interior of said chamber and adapted for connection with the containers of said biological material, a plurality of trays each located adjacent one of said outlets and adapted to support one of said containers and a refrigerant in contact with said container, and vacuum-producing means communicating with the interior of said chamber for the removal of air.

*Claim 14.* An apparatus providing means for treating biological material for preservation by dehydrating through the action of a regenerable chemical desiccant and vacuum and for regenerating the desiccant comprising a desiccant chamber, a regenerable chemical desiccant within the chamber, a plurality of outlets communicating with the interior of said chamber, a plurality of puncturable connectors each connected to one of said outlets, a plurality of containers for said biological material each connected to one of said puncturable connectors, puncturing means for puncturing each of said puncturable connectors in succession and for introducing sterile air into said connector and said container, and vacuum-producing means communicating with the interior of said chamber for the removal of air.

*Claim 20.* An apparatus providing means for treating biological material for preservation by dehydrating through the action of a regenerable chemical desiccant and vacuum and for regenerating the desiccant comprising a desiccant chamber, a regenerable chemical desiccant within the chamber, a plurality of outlets connected to said chamber and for use as container connectors, vacuum-producing means associated with said chamber for the removal of air, a plurality of containers for biological material each connected to one of said outlets, a hollow barrel having an opening therethrough, a regenerable desiccant in said barrel, and means for connecting said barrel to each of said outlets and to the container connected thereto so as to provide an inlet for dry sterile air to said container.

*Claim 22.* In an apparatus providing means for treating biological material for preservation by dehydrating through the action of a regenerable chemical desiccant and vacuum and for regenerating the desiccant, an air dryer and sterilizer comprising a hollow barrel, a supply of regenerable chemical desiccant located within said barrel, and a hollow needle connected to said barrel, there being an opening provided through said barrel and through said needle forming a continuous passage through said desiccant.

[4] *Claim 23.* In an apparatus providing means for treating biological material for preservation by dehydrating through the action of a regenerable chemical desiccant and vacuum and for regenerating the desiccant, an air dryer and sterilizer comprising a

hollow barrel, regenerable chemical desiccant in said barrel, a pointed hollow element mounted on said barrel, there being an opening provided through said barrel and through said element forming a passage through said desiccant, and detachable means securing said element to said barrel.

*Claim 24.* In an apparatus providing means for treating biological material for preservation by dehydrating through the action of a regenerable chemical desiccant and vacuum and for regenerating the desiccant, an air dryer and sterilizer comprising a hollow barrel, regenerable chemical desiccant in said barrel, porous filtering means in said barrel, and a hollow needle mounted on said barrel, said barrel and said needle having openings through the walls thereof providing a throughway through said desiccant.

*Claim 25.* A process of treating and packaging biological material for preservation which comprises, filling biological material into a receptacle adapted to serve as a final container for the biological material for protection, distribution and restoration for use, reducing the temperature of the material to not more than minus 10°C., desiccating the material at not more than minus 10°C. by means of a solid, porous, regenerable chemical desiccant and sub-atmospheric pressure until the desiccation is as complete as is possible to obtain at the aqueous tension differential between minus 10°C. and the temperature of the desiccant, raising the temperature of the material very gradually to room temperature at such a rate that the temperature at any time is never much above that necessary to produce a small further differential in aqueous tension, and sealing the receptacle.

*Claim 27.* In an apparatus providing means for treating biological material for preservation by dehydrating under vacuum, an air dryer and sterilizer comprising a hollow barrel, regenerable chemical desiccant in said barrel, porous filtering means in said barrel, and a hollow needle mounted on said barrel, said barrel and said needle having openings through the walls thereof providing a throughway through said desiccant.

*Claim 28.* The method of treating and packaging biological material for preservation which comprises, filling at least one clinical dose into a receptacle to not more than 75% of its capacity, said receptacle being suitable as a final container adapted for protection, distribution and restoration for use of contents, dehydrating the biological material by sublimation through the action of (1) a solid, porous chemical desiccant heat-regenerable without fusion and (2) vacuum until dry, while maintaining the material frozen by the auto-refrigeration of the sublimation, and sealing said receptacle to produce a final package ready for storage or distribution.

[5] *Claim 34.* A process of treating and packaging biological material for preservation which comprises, filling biological material into a receptacle adapted to serve as a final container for the biological material for protection, distribution and restoration for use, reducing the temperature of the material to not substantially more than —10°C., desiccating the material at not substantially more than —10°C. under sub-atmospheric pressure until the desiccation is substantially complete, and sealing the receptacle.

*Claim 35.* The method of treating and packaging biological material for preservation which comprises filling at least one clinical dose into a receptacle to not more than 75% of its capacity, said receptacle being suitable as a final container adapted for protection, distribution and restoration for use of the contents thereof, dehydrating the biological material by sublimation through the action of (1) a solid, porous chemical desiccant heat-regenerable without fusion and (2) vacuum until dry, and sealing said receptacle to produce a final package ready for storage or distribution.

*Claim 36.* An apparatus providing means for treating biological material for preservation by dehy-

> drating through the action of a regenerable chemical desiccant and vacuum and for regenerating the desiccant comprising a desiccant chamber, a solid, porous, regenerable chemical desiccant within the chamber, a plurality of outlets suitable for use as container connectors communicating with said chamber, a plurality of valves each controlling the communication of one of said outlets with said chamber, and vacuum-producing means communicating with the chamber for the removal of air.
>
> *Claim 39.* An apparatus providing means for treating biological material for preservation by dehydration under vacuum comprising means defining a chamber, drying means disposed within said chamber for removing water vapor from air within said chamber, a plurality of outlets suitable for use as container connectors communicating with said chamber, means controlling the communication of said outlets with said chamber and vacuum producing means communicating with the chamber for the removal of air therefrom.

IV. The defendant, Conway P. Coe, Commissioner of Patents of the United States, by the aforesaid decision of the Board of Appeals of the United States Patent Office, has refused and still refuses to grant Letters Patent of the United States to the plaintiff, F. J. Stokes Machine Company, as assignee of said Earl W. Flosdorf, Charles J. Westin and Francis Joseph Stokes, Jr., on their said ap- [6] plication, serial No. 219,858, including the eighteen claims recited in paragraph III hereof.

V. The said decision of the Board of Appeals was rendered on or about July 16, 1942, and a supplemental decision on petition for reconsideration was rendered on or about September 26, 1942, both within six months last past, and no appeal has been taken from said decision of the Board of Appeals to the Court of Customs and Patent Appeals, and no such appeal is pending or has been decided.

WHEREFORE, the plaintiff, F. J. Stokes Machine Company, demands that this Honorable Court authorize the Commissioner of Patents to issue to plaintiff Letters Patent of the United States containing the eighteen claims set forth in paragraph III hereof, upon the filing in the United States Patent Office of a certified copy of such adjudication, and upon compliance with the requirements of law for such cases made and provided.

F. J. STOKES MACHINE COMPANY,

By CLARENCE M. FISHER,
Attorney for Plaintiff,
National Press Building,
Washington, D. C.

# AMENDMENT TO BILL OF COMPLAINT

(Filed November 12, 1942)

[7] IN THE DISTRICT COURT OF THE UNITED STATES FOR THE DISTRICT OF COLUMBIA

Civil Action No. 17,515

F. J. Stokes Machine Company,
Plaintiff,
*v.*
Conway P. Coe, Commissioner of Patents,
Defendant.

Now comes the plaintiff, by his attorney, and amends the Bill of Complaint filed herein, the Answer of the defendant not having been filed, as follows:

Paragraph III, line 2, change "eighteen" to — sixteen —.

Same Paragraph, delete claims 25 and 34.

Paragraph IV, line 8, change "eighteen" to — sixteen —.

In the prayer for relief, the WHEREFORE clause, at the end of the Bill of Complaint, line 4, cancel "eighteen" and insert — sixteen.

F. J. Stokes Machine Company,
By Clarence M. Fisher,
Attorney for Plaintiff,
834 National Press Building,
Washington, D. C.

November 12, 1942.

Service of a copy of this Amendment to the Bill of Complaint is hereby acknowledged.

W. W. Cochran,
Attorney for Defendant.

## ANSWER TO THE COMPLAINT AS AMENDED.

(Filed December 18, 1942)

[8] IN THE DISTRICT COURT OF THE UNITED STATES FOR THE DISTRICT OF COLUMBIA

Civil Action No. 17,515

◆

F. J. Stokes Machine Company,
Plaintiff,

*v.*

Conway P. Coe, Commissioner of Patents,
Defendant.

◆

W. W. Cochrane, Solicitor,
United States Patent Office,
Washington, D. C.,
Attorney for Defendant.

To the Honorable the Justices of the District Court of the United States for the District of Columbia.

I. Defendant admits the allegations of paragraph I.

II. He is without knowledge or information sufficient to form a belief as to the truth of the allegations of paragraph II.

III. He admits the allegations of paragraph III.

IV. He admits the allegations of paragraph IV, but states that plaintiff is not lawfully entitled to receive a patent containing any of claims 1, 2, 3, 4, 5, 13, 14, 20, 22, 23, 24, 27, 28, 35, 36 and 39 of the application of Earl W. Flosdorf, Charles J. Westin and Francis Joseph Stokes, Jr., Serial No. 219,858, entitled "Biological Apparatus, Containers and Methods", for the reason that claims 1, 2, 3, 22, 23, 24 and 27 of said application are directed to species of the alleged invention different from the species elected for prosecution by said applicants in said applica- [9] tion, for the reason that claims 14, 20, 22, 23 and 24 of said application are for unpatentable aggregations of the subject-matter disclosed therein, and for the reason that claims 4, 5, 13, 14, 20, 28, 35, 36 and 39 of said application are unpatentable in view of the following prior patents and for the reasons given in the statement of the examiner in answer to the appeal and the decision of the Board of Appeals, copies of which will be furnished at the trial:

| | | |
|---|---|---|
| Hart et al., ........... | 653,555, | July 10, 1900, |
| Morel, ................ | 864,978, | Sept. 3, 1907, |
| Hammond, ........... | 1,887,349, | Nov. 8, 1932, |
| Elser, ................ | 1,970,956, | Aug. 21, 1934, |
| Reichel, .............. | 2,066,302, | Dec. 29, 1936, |
| Moore et al., .......... | 2,083,732, | June 15, 1937, |
| Flosdorf, ............ | 2,163,996, | June 27, 1939, |
| Krause, British, ...... | 12,642, | of 1905. |

Profert of copies of these patents is hereby made.

V. He admits the allegations of paragraph V.

W. W. Cochran,
Solicitor, U. S. Patent Office,
Attorney for Defendant.

December 18, 1942.

I hereby certify that a copy of this Answer to the Complaint as Amended was mailed today, December 18, 1942, to the attorney for plaintiff, Clarence M. Fisher, National Press Building, Washington, D. C.

W. W. Cochran,
Solicitor.

# AMENDMENT TO THE ANSWER TO THE COMPLAINT.

(Filed December 22, 1942)

[10] IN THE DISTRICT COURT OF THE UNITED STATES FOR THE DISTRICT OF COLUMBIA

Civil Action No. 17,515

F. J. Stokes Machine Company,
Plaintiff,

*v.*

Conway P. Coe, Commissioner of Patents,
Defendant.

W. W. Cochran, Solicitor,
United States Patent Office,
Washington, D. C.,
Attorney for Defendant.

To the Honorable the Justices of the District Court of the United States for the District of Columbia.

With the annexed written consent of plaintiff, defendant hereby amends his Answer, filed on December 18, 1942, by striking out paragraph II thereof and inserting in lieu thereof the following paragraph:

II. He admits, for the purpose of this action, the allegation of paragraph II.

(Sgd.) W. W. Cochran,
Solicitor, U. S. Patent Office,
Attorney for Defendant.

December 21, 1942.

I consent to this amendment.

C. M. Fisher,
Attorney for Plaintiff.

# FINDINGS OF FACT AND CONCLUSIONS OF LAW.

[12] IN THE DISTRICT COURT OF THE UNITED STATES FOR THE DISTRICT OF COLUMBIA

CIVIL ACTION No. 17,515

F. J. STOKES MACHINE COMPANY,
Plaintiff,
*v.*
CONWAY P. COE, Commissioner of Patents,
Defendant.

## Findings of Fact.

1. This complaint is brought under the provisions of Sec. 4915 R. S. (U. S. C., title 35, sec. 63) and it was sought therein to have this Court adjudge that the plaintiff, F. J. Stokes Machine Co., is entitled to have issued to it on the application of its assignors Earl W. Flosdorf, Charles J. Westin and Francis Joseph Stokes, Jr., Serial No. 219,858, filed July 18, 1938, a patent containing claims 1 to 5, 13, 14, 20, 22, 23, 24, 27, 28, 35, 36, and 39 of said application.

2. At the trial all the claims were withdrawn except 28, 35, and 39.

3. The Flosdorf et al. application discloses a process of, and apparatus for, treating and packaging biological material such as sera, guinea pig complement, vaccines, etc. In carrying out this process at least one clinical dose of the biological material is placed in a receptacle

[13] which is suitable as a final container for protection and restoration for use of its contents and having an exhaust tube connecting said receptacle to a vacuum apparatus containing a regenerable chemical desiccant, preferably a form of calcium sulphate known by the trade name "Drierite", then freezing the biological material into a solid by rapidly lowering the pressure, dehydrating the material by sublimation through the action of the desiccant until dry, closing the connection between the apparatus and the receptacle, piercing the connector and admitting sterile air to the receptacle and sealing off the exhaust tube.

4. The Flosdorf patent No. 2,163,996 discloses a process and apparatus treating biological materials, of the same type as that disclosed in the application involved herein. In the apparatus disclosed in this patent, a condenser is employed for converting the water vapor given off by the biological material into ice.

5. The patent to Elser No. 1,970,956 and the patent to Reichel No. 2,066,302 each shows a process for the desiccation of biologicals, in which a condenser is employed for the same purpose.

6. The patent to Moore et al. No. 2,083,732 discloses the use of solid, porous absorbents such as activated allumina, adsorbent gels and the like to remove moisture from air and gases for various technical and industrial uses.

7. The patent to Hammond No. 1,887,349 discloses a desiccating agent which may be either sulphate of lime or calcium sulphate, the latter being the material which [14] is designated in the Flosdorf et al. application as "Drierite" and states that this material is a desiccating agent of the highest efficiency.

8. It was not invention to use a chamber containing a desiccating agent, such as disclosed in the patent to Hammond and in the patent to Moore et al., *in lieu of the condenser* of the Flosdorf patent or that of the Elser or Reichel patents.

9. It was not invention to provide the device of the Elser patent with means controlling the communication between the tubes *a* and the air drying chamber 7 thereof.

10. Claims 28, 35, and 39 of the Flosdorf application involved herein define no invention over the prior art.

## Conclusions of Law.

1. Plaintiff is not entitled to a patent containing claims 1 to 5, 13, 14, 20, 22, 23, 24, 27, 28, 35, 36, and 39 or any one of them.

2. The complaint should be dismissed with costs against the plaintiff.

DANIEL W. O'DONOGHUE,
Justice.

February 7, 1944.

## JUDGMENT.

[15] IN THE DISTRICT COURT OF THE UNITED STATES FOR THE DISTRICT OF COLUMBIA

Civil Action No. 17,515

F. J. Stokes Machine Company,
Plaintiff,
*v.*
Conway P. Coe, Commissioner of Patents,
Defendant.

This action came on to be heard at this term and thereupon, upon consideration thereof, it is, this 7th day of February, 1944,

Adjudged that the complaint in this case be, and the same hereby is dismissed with costs against the plaintiff.

Daniel W. O'Donoghue,
Justice.

Approved as to form:

C. M. Fisher,
Attorney for Plaintiff.

## TESTIMONY OF EARL W. FLOSDORF.

[25] Earl W. Flosdorf was called as a witness for and on behalf of the plaintiff and, having been first duly sworn, was examined and testified as follows:

Direct Examination by Mr. McLean:

Q. Will you state your name? A. Earl W. Flosdorf.

Q. Where do you live, Mr. Flosdorf? A. Lansdowne, Pennsylvania.

Q. You are one of the applicants in the application involved in this suit and the Earl W. Flosdorf named there? A. I am.

Q. Will you tell us what your education in general has been, so far as your college work is concerned? [26] A. Bachelor's degree from Wesleyan University in 1925, then I went to Princeton for three years and received my master's degree at Princeton in 1928, and Ph.D. degree in 1929.

Q. What general type of study did you pursue? A. Chemistry, various branches of chemistry.

Q. What has been your occupation since? A. I taught chemistry for three years at Haverford College. Then I went to the University of Pennsylvania as a Fellow in medicine, with the National Research Council, for one year, carrying on through Research Associate, and as assistant professor in the medical school.

Q. You are now with the University of Pennsylvania? A. Correct.

Q. Do you have any other position at the moment? A. I am also director of research at the F. J. Stokes Machine Company.

Q. What was the occasion of your first introduction to the desiccation of biologicals from the frozen state? A. Dr. John Reichel, who is director of research of a biological laboratory, had been working on a process of desiccation which would be applicable to a wide variety of products. He came to us at the university where I was working with Dr. Mudd and sought our assistance in connection with certain features of this process where he was encountering difficulty.

Q. Would you tell us what this process of desiccation was as he had it? A. Briefly, it was one of taking the product to be preserved and freezing it in a large bulk type
[27] of container, freezing it at low temperatures and then placing it under a high vacuum with a low temperature condenser adjacent to the flask of frozen material and then to the condenser was attached a line leading to a high vacuum pump. Heat was introduced into the product being dried while it was maintained in a frozen condition, in order to cause rapid evaporation or sublimation of the product.

Q. What do you mean by sublimation, Doctor? A. Sublimation is similar to boiling. In certain respects it is different in that the material is solid instead of liquid when it is being sublimed and the product passes directly from the solid state into the vapor state, whereas in boiling it goes from the liquid state into the vapor state.

Q. You refer to heating it during this operation. What is the purpose of that? A. Even though the material is being sublimed, you must supply latent heat in order to cause the product to evaporate and pass into the vapor state.

Q. You refer to having a vacuum pump attached to the condenser. What was the purpose of the vacuum pump? A. In order to hasten the reaction or in order to hasten the sublimation process whereby the water vapor must come off a frozen product rapidly and pass over to the condenser and be condensed. You must have the entire system vacuumed to get practically all of the air molecules out of the air so that the water molecules may move very rapidly.

Q. What is the essential requirement from the standpoint of vacuum to have rapid sublimation? A. You must have a very low pressure over the product. In other words, [28] the vapor pressure of the frozen material is very low. Around, say, 5 degrees below freezing, the vapor pressure on the water is 3 millimeters as compared to atmospheric pressure of 760 millimeters, so that you must have pressure much below that and also temperature lower than that. The pressure must be reduced. For example, at minus 80 degrees centigrade, the vapor pressure is 1/1000 millimeters as compared with 760 millimeters atmospheric pressure in this room right now.

Q. Would you mind telling us what you mean by vapor pressure? A. Vapor pressure of the solid product is a measure of the tendency of the water molecules to pass from the solid state into the vapor state.

Q. Could you tell us something about the volume of the vapor that is evolved in that type of process? A. That is rather phenomenal. When the solid ice or frozen material passes from the solid state into the vapor state there is a tremendous increase in volume. One liter of frozen material turns into 4½ millions of liters—in other words, it is an expansion of 4½ million times. That is terrific, of course.

Q. How did Reichel get rid of that water vapor in the process? A. He used a low temperature condenser.

Q. And all that water vapor was condensed in this condenser? A. Yes.

Q. And then after that he had a vacuum pump? A. He [29] had a vacuum pump to remove the air from the system. The condenser pulls over the water vapor after the vacuum pump exhausts the air from the system. The condenser does not become operative until the vacuum pump removes the air first.

Q. Is it necessary to keep the material frozen during this whole process? Or, what are the limitations in that respect? A. It must be kept frozen for several reasons. Unless the material is kept frozen deterioration of the biological products occurs. They decompose very rapidly, so that they must be kept frozen for that reason, and the second reason is that unless they are kept frozen the dried product is hard and amberlike and does not redissolve in water again when it has to be used.

Q. You referred to supplying heat to the material which is being dried during the process. I wish you would explain a little more why that does not raise its temperature and make it melt. A. Under the conditions of the high vacuum, when the air is pumped out of the system the water from the frozen serum or whatever it is turns into vapor and passes over the condenser very rapidly. You have evaporation there as the water sublimes and that is evaporation accompanied by cooling, just as all evaporation is accompanied by cooling, so that we are using the latent heat of sublimation, so that when you apply heat to the frozen product to dry it you are having a balance between the heat being supplied from the outside and the heat which is being used up in evaporation.

[30] Q. What happens if you don't supply the heat fast enough? A. The process will gradually slow up and it cannot proceed unless you do supply heat if the process is to be carried out in any reasonable length of time, you must supply heat and supply it rapidly.

Q. How dry do you get this material? How much of the water present do you remove? A. Practically all of it, 99.95 percent of the water is removed, which leaves the final moisture content of the product less than 1 percent. There seems to be a little inconsistency there. 99.95 percent of the water removed leaves 5/100 of the original water left in, but in terms of final solids that represents about 1 percent of the solid weight.

Q. Supposing you do not reduce the water content to around 1 percent, what sort of a product do you get? A. It lacks in stability and defeats the purpose of drying it. As a matter of fact, if the moisture content is not reduced to such a low level the product would be less stable than the original liquid. Take, for example, diphtheria antitoxin, which is one of the more stable of the biologicals, yet that deteriorates much more rapidly if it is improperly dried than if it is kept in the original liquid.

Q. Can you tell us the difference between 1 percent and 5 or 6 percent moisture in a product by the appearance or feel of it? A. There is no way at all. You must run an analysis. The National Institute of Health has set up in the case of blood plasma methods for analysis. It is one of the most difficult things to determine whether [31] moisture is down under 1 per cent.

Q. What does the final product look like? A. It is a highly porous, friable solid.

Q. I have here a product which is labeled "Complement". Can you tell us what that is? A. It is a serum prepared from guinea pigs. Complement happens to be one of the components which is present in large amount in the blood of guinea pigs and complement is used in the Wassermann reaction in hospitals and such things.

> Mr. McLean: I would like to offer this in evidence as Plaintiff's Exhibit No. 2. Your Honor might care to look at it. It is the small bottle with the dried material in it, as the product that we are talking about.
>
> (Bottle containing dried material labeled "Complement" was marked Plaintiff's Exhibit 2, and received in evidence.)

By Mr. McLean:

Q. Why cannot you keep complement in the liquid state in which it is produced? A. It begins to deteriorate as soon as the blood is removed from the animal. After about 24 hours it has deteriorated to the point it is no longer usable in the Wassermann reaction.

Q. How long is this material good for? A. We have had complement fully active after five years in the dried state.

Q. I notice on that container in Plaintiff's Exhibit 2 there is quite an elaborate closure with a stopper inside of the neck and a glass seal over it. What is the purpose of that? A. That, again, is related to the moisture content [32] of the product. I have indicated that the product must be gotten very dry. That is not the end of the story. After you get it dry you must keep it dry. The ordinary rubber stopper in a bottle is inadequate to keep the water vapor in the atmosphere from passing through that stopper, so that the only way you can have a satisfactory reduction of that loss for a long period of time is to have a hermetically sealed container. The rubber stopper down inside the stem is for the purpose of making it easier to use the product. When a person uses it they simply snap off that glass stem above the stopper; then by use of the syringe and needle the water can be added to the product through that rubber stopper and the product reconstituted. It is important to do it that way because these products are biological products, products that are very rapidly contaminated by bacteria, so that extreme precaution must be taken not only to hold out the moisture until it is to be used, but in order that when the product is used you can get the water back in without contaminating the product.

Q. Well, now, you have described generally the process which Dr. Reichel had at that time, in which he desiccated from the frozen state and eliminated water vapor by condensing it in the cold condenser. Will you describe—I think you call it the cryochem process, do you not? A. Yes.

Q. Which is involved in the application in suit. A. The product is frozen under conditions similar to that which Reichel describes and we use a highly porous chemical substance known as drierite, which is chemically calcium [33] sulfate.

Q. Is this the product (handing bottle to witness)? A. Yes, sir; that is right.

Mr. McLean: I offer this in evidence as Exhibit 3.

The Court: It is received as Plaintiff's Exhibit 3.

(Bottle containing material labeled Drierite was marked Plaintiff's Exhibit 3 and received in evidence.)

By Mr. McLean:

Q. Will you proceed to describe the process, Doctor? A. Under the conditions of the high vacuum which exist in the system the water vapor which sublimes from the frozen product passes over into that chemical. That chemical under these conditions of high vacuum has a tremendous affinity for water. That was one of our major problems to determine that that could occur and that chemically would take up the water under those conditions. It is imperative that the substance absorb or take up that water rapidly because, as we indicated before, in order to dry the product you have to put heat into it and put it in rapidly in order to drive out the water vapor, in order to make the sublimation take place rapidly. The faster one puts in the heat to drive through that sublimation, the more efficient this chemical must be. This chemical absorbs that water vapor as rapidly as it comes over and carries the process until it is completed, as just described.

Q. You referred to "under those conditions." Would you mind stating in general what those conditions are in which this desiccant must absorb these large quantities of water vapor? A. The desiccant must absorb the water vapor under the conditions of high vacuum. Furthermore, you must remember that desiccant is present in a [34] high vacuum system where you have large openings and a communicating passageway between the position where the desiccant is located and where the frozen product is located. They must be adjacent to each other to have it operative. Under these conditions one must have a desiccant which definitely can cause no harm to the product being dried. If any of the desiccant would get over into the product it would be very dangerous if the desiccant would have any deleterious effect on the product, these products being very delicate, it is essential that the desiccant be inert except as far as taking up the water. That must be its sole function.

Q. We will come back to that. I would like to go back to the time when Dr. Reichel first brought this process to the attention of you and your associates—where was it, at the University of Pennsylvania? A. That is right.

Q. What was he concerned with at that time? A. Well, he had succeeded in drying the product with the aid of these low temperature condensers, the product being dried in bulk. He was concerned because after he had dried the product he didn't know what to do with it. The products he was drying were for injection into human beings, and, of course, in the veterinary field in animals. He had the problem of getting this product into the containers which would be suitable for use by the physician or veterinarian, and that presented an insurmountable problem because he could not get his dry product into the container without danger of contamination. Secondly, he could not get it [35] into the container without having the product pick up some moisture from the atmosphere. In certain experiments we have carried out it is shown these dry products will absorb water vapor from the air at the rate of about 1 percent a minute. Although these products are delicate, they are highly active and the quantities measured into these individual containers must be measured very accurately. You must be careful not to have an overdose, or too little. To weigh the product and get it transferred without taking up bacteria and moisture proved to be impossible, so that we then developed the method of drying in the final container in which the product is to be stored and used. That is the method which is used today generally for blood plasma and all other applications of this process.

Q. You refer to contamination. Why not just sterilize the product after it was transferred? A. There is no way of sterilizing it. You can sterilize it when it is liquid by filtering it. That is the standard way of sterilizing liquid serums, et cetera, but with a dried product you cannot do that. You cannot heat the product, even though the product is much improved in stability as a result of this process, you cannot possibly heat it to a temperature of 200 degrees centigrade.

Q. You are familiar with the Reichel patent, number 2,066,302, which is part of Defendant's Exhibit 1. Does that contain a fair description of the process Reichel had at that time? A. Yes.

Q. In the course of this development and work which you did at that time, did you make any other improvements [36] in this procedure except the suggestion of carrying out the process in the final container? A. Yes. We developed methods of actually handling the containers and many incidental features. One rather important development was that of the secondary condenser which was interposed between the condenser which Reichel used and the vacuum pump.

Q. Some of these improvements which you have made at that time were the subject matter of patents? A. Yes.

Q. Of which patent No. 2,163,996, which is also included in Defendant's Exhibit 1, is one? A. Yes.

Q. Will you tell us what the invention in that patent was, if you will? Do you have a copy of that? A. Yes, sir. This patent discloses an apparatus which is suitable, particularly suitable, for use in research laboratories and general laboratory purposes.

Q. Why should anyone want one of these for a research laboratory, Doctor? A. This particular apparatus which is referred to here has probably resulted in as many major developments in medical research as any other single piece of equipment in recent times. It has opened up new methods of handling products which could not be handled in any other way. For example, viruses, of which relatively little is known. That is just one of the many products which can be handled by this apparatus, and the product is dried and the laboratory worker is able to use it and work with the product under conditions which were never possible [37] before.

Q. Well, go ahead. A. The condenser, as I have referred to a few moments ago, the secondary condenser is particularly of value by placing it between the first condenser, which is Reichel's condenser, and the vacuum pump. As I mentioned before, it is the condenser in Reichel's vacuum pump which takes the water vapor from the product being dried. A small amount of that water vapor, however, passed over with the air to the vacuum pump. They are rather minute amounts which are contaminating the air, but they do not affect the process particularly. What is serious about it, however, is that the vacuum pumps which are used are sealed with oil; that water vapor accumulates in the oil and very soon the vacuum pump is no longer able to produce the vacuum which is required. This dries out the air which goes to the vacuum pump.

Q. What was the common practice in laboratories using vacuum pumps to protect them from water vapor? A. They used phosphorus pentoxide and sulfuric acid. The air which passed to the vacuum pump was made to go over a tube of phosphorus pentoxide, and in that way—

Q. Was that the common expedient in laboratories? A. Yes, that has been used for years.

Q. What sort of material is phosphorus pentoxide? By the way, is this some of it (indicating)? A. It is an acid without water. It is a solid powder. That is, the acid is very strong. The product is very light in physical form, and fluffy.

Q. It would make a pretty good smoke screen if you [38] dispersed it in that way? A. Yes. As a matter of fact, that has been one of the original types of smoke screen used to produce that over the ocean.

Q. Easily blown around? A. Yes. If you shake that container you see that very clearly, the way it tends to go up in a cloud.

> (Bottle described as containing phosphorus pentoxide was marked Plaintiff's Exhibit 4 and received in evidence.)

By Mr. McLean:

Q. What would happen if some of that was brought into contact with one of these biologicals? A. It would be very serious. It would react with the product. You must remember that these products are very delicate and they are injected into human beings. A strong acid of this sort is able to cause very slight changes in the product, small in amount, if not much of the product gets over. Of course, if a lot goes over there would be major changes, but what is dangerous is small amounts getting over, because there would be no way of knowing that the particular container of the product had a little bit in it and changing it slightly—the nature of those changes is known as denaturation—and such products when injected into human beings is very dangerous. Sometimes the first injection is not so serious. Sometimes the harm is not caused until the second dose is given, and frequently death results from the injection of such types of products.

Q. How much of this phosphorus pentoxide might it [39] take to do that sort of thing? A. Just a few granules. It is well known that to determine the presence of a small amount, the only way you can do it is to use animals for experimentation and find out if you do get such a reaction occurring.

Q. Is there any way to tell before you inject it into anybody? A. The only way you can tell is to inject it and see what happens. In other words, you just cannot do it as a practical matter.

Q. Were objections to desiccants of that type recognized at that time? A. Yes, they were well known. Reichel described that in his patent.

Q. Would you show us what Reichel had to say about it in patent 2,066,302? A. He said:

> "It will also be noted that the material is protected, during the removal of water therefrom, while in a solid frozen state, from contact with the atmosphere and from contact with or use of any of the known desiccating materials."

Q. Now, you have also referred to Elser. Does he have something to say on the subject? The Elser patent is number 1,970,956, which is also part of Defendant's Exhibit 1. A. Yes. Elser said:

> "It may here be noted that this system neither depends upon nor contemplates chemical absorbents for performing the desiccation. Although such agents, as for example, phosphorus pentoxide [40] have a powerful affinity for water, and a very low vapor pressure, nevertheless they (or perhaps, certain impurities thereof) are volatile to the extent of their vapor pressures, and may bring about

> changes in the serum such as to defeat the purpose of the invention. This has been demonstrated to be a fact.''

Q. Now, you have been talking about phosphorus pentoxide. Sulfuric acid is also a known desiccant? A. Yes.

Q. How about it? What sort of a product is it? A. It would be even more serious than phosphorus pentoxide. It is a liquid. I suppose the best example of that is the acid which is in your storage battery in your car, although the storage battery of the automobile is diluted down somewhat, but for this purpose you have to use the strongest and most concentrated solution of it, and being a liquid it would be impossible to have that in such close proximity to these dried biologicals.

Q. I want you to refer to your patent No. 2,163,996, if you will, toward the bottom of the second column on page 1, where you say—you discuss the secondary condenser and say it serves as a protection for the vacuum pump, as you have explained: ''making unnecessary the provision of an absorbent tube of dehydrating material such as P2 05 between the condenser and the pump.'' What does that statement mean, Doctor? A. Well, in this we use a low temperature condenser like Reichel used to remove water vapor rapidly from the material being dried. A very small amount of that water vapor, as I mentioned before, will be carried by the air over to the vacuum pump. It is carried [41] by the air into the vacuum pump and soon the oil of the vacuum pump has a lot of water mixed up in it and is no longer efficient, and you are not able to keep a proper vacuum. Phosphorus pentoxide could be used for that purpose because it is not near the material being dried. It is over on the other side of the condenser, which is in the air line to the pump. This secondary condenser avoided the necessity of using things of that sort. It made a very satisfactory, convenient, and efficient way of protecting the vacuum pump.

Q. In 1935—I think that is the date of the filing of the application—you regarded it as an advantage that you could avoid the inclusion of a desiccant in that system by the provision of a secondary condenser. A. Yes; even though it would be still far from the product being dried, it was still an advantage just to keep out the phosphorus pentoxide entirely.

Q. There has been some reference to this desiccant which you use. Was it a known product? A. Yes.

Q. I want to refer you to the Hammond patent, number 1,887,349. Is it the product described in that patent? A. Yes, that is right.

Q. Can you find anything in that patent which suggests anything about the behavior of this material under a high vacuum? A. No, nothing at all. There was nothing known about it.

Q. As far as you know, was anything known about that [42] prior to 1938? A. Definitely not. As a matter of fact, Hammond was very much surprised and pleased to learn of the fact we had opened up a brand new use for his product, and still today takes around one of these little machines with him to all the exhibits and so on. Hammond manufactures this material and shows it as some new developed use for his product, and is very happy about it.

Q. Are there any desiccants which are used for absorbing water in various operations that won't operate under a high vacuum? A. Oh, yes. Sodium carbonate, copper sulfate, sodium sulfate, many of those desiccants, as a matter of fact, they lose their water under high vacuum under these conditions.

Q. Are they porous? A. Yes.

Q. I want you to refer to the Krause (British) patent, No. 12,642, also included in Defendant's Exhibit 1, and tell us briefly what the process of that patent is. A. Krause describes freezing such things as meat and keeping them in an ice box or cold vault below freezing temperatures, 20 degrees minus centigrade, and then he places the desiccants in the ice box or drying place. The air around the ice box allows the desiccant to take up the moisture and form what you call a partial vacuum. I say partial vacuum because it is 20 millimeters; whereas in this process we operate at a fraction of 1 millimeter. It is necessary to operate at that pressure for it to be operative.

Q. What happens if the pressure goes, say, above a [43] millimeter in your process? A. The material melts. The evaporation cannot take place fast enough to keep it frozen.

Q. How does Krause avoid that melting? A. He keeps it in a freezing vault.

Q. So that even if it did not evaporate at all, it would stay frozen? A. That is right, but that is not a practical way of doing it. As a matter of fact, he does not get his material properly dried. There is a fair amount of evidence in here to that effect. For example, one thing is his method of determining whether it is dry. He says you can determine whether it is dry simply by the feel. That is impossible. A product may have 20 percent moisture in it and you could not tell the difference between that and a completely dry product, simply by feeling it. Then, more than that, he states later on—

Q. Whereabouts? A. Page 4, about line 36:

> "In an atmosphere having a normal proportion of moisture the substances treated do not absorb any more moisture from the air."

That can only mean one thing. That is, they were not dry enough where they could pick up moisture from the air. In other words, if we take our product from that container and open it up and spread it out on the table, it will pick up moisture at the rate of about 1 percent a minute until it gets about 20 percent. That is as much as it can take.

Q. How stable would such a product be, referring now to biologicals, with which you are primarily familiar? A. I don't know about the stability of meat, which [44] Krause is concerned with. Meat with even less moisture than that will last about two or three weeks. Liquid complement is good not over 24 hours. Those products properly dried will last as long as five years. We have had some products with only 5 percent moisture and in a matter of weeks the potency had gone down to a point where it was no longer usable.

Q. Now, Doctor, I wish you would refer to the Morel patent, No. 864,978, which is "B" in Defendant's Exhibit 1, and tell us what type of process that patent describes. A. That is something entirely different. The only thing similar about it is that Morel does freeze his product before drying it, but then he melts it again before he dries it. He describes on the first page the process he has carried out. In the first place, it has a very high pressure, 60 millimeters, which is even higher than that of Krause. Then in the second column, around line 71, he says:

> "As soon as a portion of this finely divided ice melts, the liquid is instantaneously evaporated by the action of the vacuum."

He apparently freezes it for some reason which may have some value in connection with gluten, but then to dry it, it was melted again. Of course, that would not work at all with our products.

Q. Do you find in your operations that some of the material melts for one reason or another? A. Yes.

Q. What do you do with the product? A. It must be thrown out. In other words, if something goes wrong and [45] the product melts, we speak of it as fusing. The potency of that would go off. Furthermore if you continue the drying you get a product which is amberlike wherever it is melted and that won't dissolve properly, so that this would not be operative at all, with biological products such as we are dealing with.

Q. Is this cryochem process we are talking about in use today? A. Yes, there are over one hundred installations throughout this country and a good many countries of the world.

Q. Is this a list of the installations (handing paper to witness)? A. Yes. This speaks for itself.

Q. You prepared it at my request? A. That is right. There are installations which have actually been shipped around and are in use in Africa, India, and I think there is one in the Philippines which unfortunately went to Japan.

> (Document entitled "List of installations of cryochem apparatus" was marked Plaintiff's Exhibit 5, and received in evidence.)

By Mr. McLean:

Q. What is the particular virtue of this process, as you see it, as compared, for example, with this cold condenser of Reichel's? A. It has many advantages. In the first place, it can be used where dry ice is unavailable; secondly, it is more economical than the use of dry ice in certain types of operation. For example, where products which are small in volume for a single dose—blood plasma, for example—you have 300 to 600 c.c. as the size of a single dose. [46] For things like vaccines and viruses, it is being used for that today for a number of viruses. It is being used for the production of a vitamin B material for intravenous use which is prepared in only c.c. amounts. In things of that sort it is pretty valuable and convenient.

Q. I have here some pictures. Will you tell us what they are, Doctor? A. Yes, sir. 176-2 is a machine which is in operation in Kenya, South Africa. 176-e is in operation in Liberia, in Africa. 176-AA-16 is in operation—I am not sure where this particular machine went, but there are about eight like it in operation in this country today. Oh, yes; this particular machine is now at the Veterinary Hospital in Staten Island, New York. 376-AA-S-1 is at the Lakeside Laboratory in Milwaukee, Wisconsin. 376-e is at the Children's Hospital in Philadelphia, producing serum for use in children's diseases.

Mr. McLean: I would like to offer these photographs in evidence.

The Court: They may be received.

(Photographs described as installations of cryochem apparatus, were marked Plaintiff's Exhibit 6 and received in evidence.)

Mr. McLean: I would like to also offer in evidence an article from the "Annals of Internal Medicine", of August 1940, pages 201 to 214, by Hill and Pfeiffer, to show independent comment on these processes and the state of the art. I have here both the original reprint and the photostat, whichever is more convenient I will offer them for your Honor to look over.

(Article from "Annals of Internal Medicine" was marked Plaintiff's Exhibit 7 and received in evidence.)

## FROM PLAINTIFF'S EXHIBIT 1.

### Specification and Drawings of Flosdorf, et al. Application Serial No. 219,858, Filed July 18, 1938.

This invention relates to new and improved methods and devices for the efficient concentration, drying and preservation of biological substances such as sera, guinea pig complement, vaccines, breast milk, etc., and, more particularly, to an improved process of treating the biological material during dehydration which may include such steps as de-gassing, self-freezing, dehydration by sublimation through the action of a regenerable chemical desiccant and vacuum.

Other features are the improvement in the apparatus for the efficient practice of the dehydration and sealing process including convenient means for the regeneration of the desiccant; and the improvement in providing suitable receptacles for use as containers for individual doses or multiples thereof during dehydration, sealing, storing, distribution and restoration of the biological material. The invention includes new and novel means for connecting the small size containers, for constricting the exhaust tubes after the admission of dry, sterile air, the re-evacuation and final sealing of the container.

The purposes of our invention agree with the features listed by Earl W. Flosdorf in his application Serial No. 131,832, filed March 19, 1937, now Patent No. 2,215,265 issued August 19, 1940. These are:

> To eliminate loss of quality;
>
> To favor retention of volatile constituents;
>
> To prevent chemical changes;
>
> To prevent multiplication of micro-organisms and changes in the product caused by such contaminants;
>
> To increase the keeping qualities for an extended period; and
>
> To improve stability.

[59] It is also the purpose to provide an article of manufacture or package consisting of a dry biological substance of superior stability in amounts suitable for clinical use, conveniently and easily restorable to original consistency by adding water, packaged in the original individual receptacle which may be of ordinary flint-glass, aseptically and hermetically sealed under vacuum.

Our invention has also for one of its purposes the provision of a simplified and improved apparatus for the efficient and economical production of packages containing biological products embodying the above-enumerated qualities. The packages may be of varying kinds, types and sizes, the general classification being as follows:

(a) An all-glass receptacle aseptically sealed ununder the original vacuum. Such a product, is for example, a test-tube confining dehydrated microorganisms.

(b) An all-glass receptacle aseptically sealed under secondary vacuum.

(c) An all-glass receptacle sealed non-aseptically under a secondary vacuum.

(d) A rubber-stoppered flint-glass receptacle aseptically or non-aseptically sealed under a secondary vacuum.

Another purpose is the simplification of the treatment of the containers or receptacles and the methods of sealing them.

Another purpose is to provide an efficient means for the economical regeneration of the desiccant.

Some of the new and novel features included in our apparatus are:

[60] A unitary vacuum-dehydrating and regenerating chamber;

*Specification and Drawings of Flosdorf, et al.*
*Application Serial No. 219,858.*

Retaining baskets and/or trays for the regenerable desiccant;

Means providing tortuous paths exposing large surface areas of the desiccant to the vapors when dehydrating and to the warm air when regenerating;

Means in the baskets or trays for testing the condition of the desiccant;

Manifolds for the freezing, dehydrating and sealing of a plurality of individual containers;

Container supporting-trays providing a maximum air circulation and heat transfer;

The method of and devices for regulating and holding the vacuum at suitable pressure for degassing and for enabling quick attainment of high vacuum for self-freezing;

Means for efficiently regenerating the desiccant;

And other features and improvements in the apparatus and devices resulting in greater efficiency and economy as shown in the appended drawings and/or hereinafter described.

The feature of having a unitary vacuum-dehydrating and regenerating chamber is of exceptional convenience and desirability in any apparatus used in biological production, operated in connection with a hospital laboratory, or in similar institutions. Heavy labor incident to the transfer of two or three hundred pounds or more of desiccant is avoided because the apparatus oven or regenerating chamber is eliminated. This also results in a considerable saving of time and reduces to a minimum [61] time-out for regenerating. Another advantage with this combination unit is that any handling of the desiccant, which would have a tendency to break up its granular form and shorten its useful life, is entirely unnecessary.

The advantages and benefits derived from the use of a direct *regenerative* chemical desiccant, as against the heretofore used expensive condensers with their also costly but necessary *low-temperature* refrigerating-mixtures as described in the applications for U. S. Letters Patent by Earl W. Flosdorf, Serial No. 131,832, filed March 19, 1937, and Serial No. 178,537, filed December 7, 1937, also apply in this case. In the above applications, the trade-name "Cryochem" was selected to identify the process wherein a regenerable desiccant is used and for the resulting product. This trade-name "Cryochem" is therefore also used in this application to distinguish from that of the "Lyophile" process and apparatus described in the applications for Letters Patent by Earl W. Flosdorf, Serial Nos. 54,148 and 54,149, filed December 12, 1935; and Serial No. 54,450, filed December 14, 1935.

It has been found that in many cases the "Cryochem" products are superior to that of the "Lyophile" product because of the vacuum-degassing, self-freezing process, which produces a complete removal of entrained gases and thereby reduces the rate of any subsequent deterioration which might be caused in the "Lyophile" product by the presence of gases, such, for example, as oxygen or carbon-dioxide. The "Cryochem" product is still further superiod to the "Lyophile" because in its preparation it is only acted upon by an aqueous desiccant and therefore only water is removed, while other volatile constituents remain. The reason for this is that in the "Lyophile" process the [62] exceedingly low temperature in the condenser withdraws not only water but any and all other volatile constituents, because the vapor pressure of all constituents is decreased to the temperature of the condenser, while with the "Cryochem" process only the vapor pressure of the water is reduced.

Furthermore, the vacuum-degassing, self-freezing process in many cases produces a fluffy frozen mass which permits more rapid removal of the water. The resulting dry product is found to be more rapidly soluble not only because of its having been dried more rapidly but also because the fluffy form is retained in the dry product to permit better contact with the water used in its restoration.

A number of desiccants are available for use in the "Cryochem" process. At present, however, the generally preferred type is that known by the trade-name "Drierite" described in U. S. Letters Patent No. 1,887,349, granted to William A. Hammond, November 8, 1932. Aside from its low first cost, it is preferred because there are no acid or other deleterious vapors present in it to affect the quality of the product.

The resulting product is a very porous solid occupying the same volume as the liquid from which it was prepared. Its content of antibodies and complement suffer no detectable loss in the processing, and the rate of subsequent deterioration is reduced to a small fraction of that of the same product in the liquid state. The porous "Cryochem" material re-dissolves with remarkable ease and completeness when distilled water is added. Typical materials which can be preserved by the "Cryochem" process are serums of all types, guinea pig complement, bacteria, viruses, vaccine, breast milk, food products and other desirable substances subject to deterioration from moisture and/or heat.

[63] The size and the shape of the containers used with the apparatus and the method of sealing have a definite effect on the resulting product. Various types of glass containers with rubber stoppers or a combination of glass and rubber are quite satisfactory for some products. For some products, however, an all-glass container is necessary. It may be of either "Pyrex" or ordinary flint-glass and the method used in sealing-off the container depends on the kind of glass used. New and novel methods for the proper and efficient sealing of the containers, whether made from "Pyrex" or flint-glass, will be described hereinafter.

For a better exposition of our invention, reference is made to the annexed drawings in which the embodiment of a suitable apparatus illustrating the principles of our invention is illustrated but we do not limit ourselves to the specific construction shown.

The drawings also include illustrations of the new and novel type of containers used in the practice of the invention.

Referring to the drawings:

Fig. 1 represents a front elevation, mostly in section, of the apparatus as in use while dehydrating.

Fig. 2 represents a front elevation, mostly in section, of the apparatus as arranged for use in regenerating the desiccant.

Fig. 3 represents a section on line 3—3 of Fig. 1 through one of the manifolds showing in an enlarged, fragmentary, sectional, front elevation the relation of parts while dehydrating.

Fig. 4 is an enlarged, perspective view of the type of basket shown in use in Fig. 2.

[64] Fig. 5 is a fragmentary, perspective view of the same basket as shown in Fig. 4, but with the test block inserted.

Fig. 6 is an enlarged, perspective view of the horizontal type of basket shown in use in Fig. 1.

Fig. 7 represents the cylindrical air duct placed in alternating baskets as shown in Fig. 1.

Figs 8 and 8*a* show an "S" connector tube and container as sterilized before filling.

Fig. 9 shows how the sterile tube and container are partly assembled before attaching.

Fig. 10 shows a filled container as attached to the manifold.

Fig. 11 shows the container with the sealing tube being restricted.

Fig. 12 shows the container with the sealing tube as finally sealed.

Fig. 12*a* represents a typical finished product.

Fig. 13 shows an "air dryer and sterilizer".

Fig. 14 shows two types of containers in multiple connection on the manifold.

Figs. 15, 16 and 17 show different types of connector tubes.

Figs. 18, 19, 20, 21 and 22 show different types of suitable containers.

Figs. 23, 25 and 24 show three typical types of stoppers.

Referring to Fig. 1, the apparatus when used for Dyhydrating consists in combination: of a vacuum-desiccant chamber 21 with one or more manifolds 23 attached to the chamber, each manifold being provided [65] with means for attachment of a plurality of containers, and a vacuum-producing means 22 also associated with the vacuum-desiccant chamber.

Referring to Fig. 2, the apparatus consists in combination, when used as a REGENERATING unit, of the desiccant chamber 21 and the air-heating and circulating device 24.

The vacuum-dehydrating chamber, containing the regenerable desiccant, may be of any suitable shape but for our purpose we prefer the cylinder-tank-type 34, provided with a dished bottom 34*a,* having an inlet-outlet 40 of ample size and suitable for connection to either the vacuum-producing means 22 or the hot air circulating unit 24. The top or open end of the chamber may be covered with a dished head 26, having provision for the attachment of a vacuum gauge 25, and arranged so it may be clamped down vacuum-tight by means of screws 30 against a gasket 29, fitted into the flange 31 of the chamber 34. To minimize the effect of the surrounding air temperature as well as to keep radiation losses down, the chamber may be surrounded or covered by some insulating material as at 35. In the side wall of the chamber, provision is made for attaching one or more manifolds 23. The connection may be either a screwed joint or the companion flange type. The chamber may be filled with any regenerable desiccant. We prefer to use "DRIERITE" because this desiccant is the most economical and appears to possess more desirable properties than any other desiccant available at present. The desiccant may be placed in the chamber in a solid mass and without any provision for the labyrinth of passages shown in the drawings. In order to obtain the greatest efficiency, however, both in the dehydrating and the regenerating cycles, we prefer to use trays or baskets [66] and support the desiccant in such a manner that large areas of it are exposed and a tortuous path provided for the vapor or the hot air as the case may be.

Two types of "dessicant baskets" are shown. A vertical type of basket is shown in Figs. 2 and 4. A horizontal type is shown in Figs. 1 and 6. Either type has proved very efficient and, in addition, it makes it easy to handle the 300/400 lbs. of desiccant when necessary to make a change.

The horizontal type of desiccant basket may be made from any suitable wire screening or perforated metal in the shape shown in Fig. 6. These baskets are usually provided with a flat bottom 71 of an outline suitable to the shape of the chamber into which it must fit and a rim 72 of 2″ to 3″ in height. It will be noticed from Fig. 1 that the baskets are not all of the same diameter but every other one is made slightly smaller as at 32, leaving an air space 36 between the rim of the basket and the chamber wall. The basket shown at 33 fits snugly, thereby shutting off the free air passage and forcing the air or vapor to pass underneath or between the baskets and/or up through or down through the perforation and voids in the desiccant. In the basket that fits snugly into the chamber, we may provide a cylindrical passage 48 in the form of a tube made from perforated metal or wire screen and place this somewhere in the approximate center of the basket before filling it with the desiccant. In the top basket the desiccant is shown at 47, and in the second basket from the top the cylindrical passage is shown at 48. An enlarged view of this cylinder is shown in Fig. 7. To support the baskets one on top of the other, bars 73 are placed across and attached to the top of the rim 72. These bars also are a convenience when lifting the baskets in or out of the chamber.

[67] The vertical basket shown in Fig. 4 may also be made from either wire screen or perforated metal and in shape to suit the chamber outline. In general, they may be spoken of as the envelope-type because they are so high and narrow with sides closed and only the top open. The particular one shown in Fig. 4 is of rectangular shape with the open top 74 terminating into the narrow end 75 and, of course, a similar bottom. Straps are provided at 77 to brace and support the flexible sides and these may be extended to the outside so as to form the spacer 76. Another type of spacer is shown at 78. Now referring to Fig. 2, these envelope types of baskets are placed side-by-side in the chamber 34 and the spacers 76 and 78, just referred to, will hold the baskets sufficiently apart as indicated at 45, to provide air passages and thus expose large areas of the desiccant to the vapor and air. The desiccant is shown at 44 within the basket 46.

The Desiccant: As mentioned above, various types of chemical desiccants are available but the type generally preferred at present is that known by the trade-name "Drierite" and described in U. S. Letters patent 1,887,349, granted to William A. Hammond, Nov. 8, 1932. This desiccant is anhydrous calcium sulfate and possesses a sufficient affinity for water, both to produce a low aqueous tension and to do it rapidly and with a sufficiently high temperature co-efficient of reversibility to be completely regenerable without vacuum. The aqueous tension of the half-hydrate which is produced is 0.004 mm. at 25° C.; this will reduce the final residual moisture content of the dried biological to the low level required. Furthermore, the degree of porosity of calcium sulfate when obtained [68] in the form of "Drierite" is so great and remains so great that the *rate* of *removal* of water vapor is more rapid than by any other means we have as yet encountered. It is a material of low initial cost and can be regenerated time and again at a minimum cost because the regeneration may be carried out under atmospheric pressure.

Calcium sulphate has no vapor pressure or volatile substance and can therefore cause no harmful effects on labile material such as has been reported with sulphuric acid or acid phosphoric anhydride. During regeneration, calcium sulfate hemi-hydrate will not lose its original porosity as calcium chloride does. Calcium sulfate has affinity for water only; this is an advantage in the case of many products, although it may occasionally be a disadvantage in certain types of work.

We do not wish to be limited to the use of calcium sulfate although at present this type of regenerable chemical desiccant appears to be the most efficient and most economical. Any desiccant that meets the following brief specifications will serve our purpose quite satisfactorily:

> A desiccant which is regenerable without vacuum, at the temperature below 350°C., yet with a very low (0.001 to 0.05 mm. Hg.) aqueous tension at 20°C., which maintains this aqueous tension until it has reacted at least to 50% of its theoretical capacity, and has a high degree of porosity and/or effective reacting surface which is not altered or rendered appreciably less efficient upon absorption of minimal amounts of water vapor; and one which does not evolve deleterious vapors during the dehydration cycle and does not fuse during the regeneration or during the desiccation cycles.

[69] The size of the VACUUM-DESICCANT-CHAMBER 21 must be large enough to hold an ample supply of desiccant or water absorbent. The amount of desiccant needed depends, of course, first, on the type used; and, secondly, on the amount of water vapor to be removed in the apparatus. Enormous volumes of water-vapor are evaporated even from a small

unit volume of a frozen substance when kept under high vacuum for an extended period of time. Sufficient absorbent material should therefore be available in the system to efficiently absorb the vapors rapidly enough so as not to interfere with or retard the evaporation. When "Drierite" is used, it has been found that the greatest efficiency is obtained by allowing 100 pounds of desiccant to every 500 milliliters. This proportion is only ¼ of the total practical capacity of the "Drierite". This proportion has been found to be the most efficient for practical operation and insures a long life of usefulness for the "Drierite". It also means that 2 liters or 4 runs of 500 ml. per 100 lbs. can be made before regeneration is necessary.

The stoichiometrical capacity of calcium sulfate ("Drierite") to react with water vapor to produce the hemihydrate is 6.6% of its weight. This theoretical capacity may be obtained. Under practical conditions, however, the rate of reaction starts to fall below the level desirable for this process after 75% of the theoretical limit has been reached. It may be noticed that among desiccants available this is a very high value. It has been found that 2 liters of serum or similar substances per hundred pounds of "Drierite" is the most satisfactory ratio in practical operation.

[70] The reaction to produce the hemi-hydrate, however, is exothermic and the maximal quantity of serum which can be dried in a single run has been found to be one-half of the total capacity; namely, one liter of serum per 100 pounds of calcium sulfate. If this quantity is exceeded, the temperature of the "Drierite" rises to the point where it can no longer react with water at a sufficiently rapid rate. Unfortunately, dissipation of heat in vacuo is very slow. It has also been found that the life of "Drierite", i.e., the number of regenerating cycles to which it may be exposed without undue loss of efficiency, is dependent upon the condition of temperature

to which it is subjected; therefore, although it will dry one-half of its total capacity on a given run, the life of the "Drierite" is lengthened and the maximum efficiency obtained if the proportion is held to the figures given above for each single run.

The desiccant chamber is therefore proportioned according to the above principle to take care of a predetermined number of manifolds and manifold outlets. It should, however, be noted that the amount of water removed per run may be as much less as desired and no greater cost incurred. It only means that so many more runs can be made before regeneration is necessary. Herein lies one great advantage of this process as compared with the "Lyophile" where low temperature condensers for the removal of the vapors are used. In the latter, the losses due to thermal leakage are large and remain constant, irrespective of how small the run volume may be. Another decided disadvantage with the "Lyophile" low temperature condensers is the fact that the losses due to thermal leakage are constant, even when the apparatus is not in use. In the "Cryochem" apparatus, the desiccant chamber is therefore so designed that the ap-
[71] paratus may be used for four or more runs without regeneration, the volume of material to be desiccated being ¼ or less of the total capacity of the "Drierite". Occasionally, if necessary, these amounts may be exceeded up to ½ of the total capacity. Another advantage to be noted is that, if the apparatus is kept completely closed between runs, there will be no deterioration of "Drierite" when not in use.

As will be noted from the above description, a definite relationship, therefore, exists between the size of the vacuum-desiccant-chamber and the number of manifolds, number of outlets on each manifold, and size of containers that can be efficiently dehydrated in the system.

Test Plugs: For convenience in testing the condition of moisture content of the desiccant, test plugs 80, Fig. 5, are provided in one or more of the baskets. They consist of a small basket made so as to conveniently fit into a space provided for that purpose in the larger basket 46. At stated intervals, these test plugs may be removed and a moisture determination made on the contained desiccant.

VACUUM REQUIREMENTS: Any reliable high-vacuum-producing means, such as the motor-driven pump 22, may be used. It is desirable to have it connected as close to the vacuum chamber 21 as practical and the ducts of ample size so that a high vacuum may be obtained quickly when needed. In Fig. 1 we have shown a duct 39 connecting outlet 40 from chamber 21 through a valve 37 to the vacuum pump 22. Valve 37 is used for two purposes: to choke down duct 39 during the de-gassing period and keep the vacuum of the system just above the frothing point of the drying substances; and to completely shut-off the pump after the high vacuum is obtained, providing the system is perfectly vacuum-tight. [72] A valve 38 is also provided. This is used to help regulate the vacuum during de-gassing and as a vacuum break to admit air to the system after the drying is completed on substances that satisfactorily may be sealed under atmospheric conditions and non-aseptically.

The pump should be of sufficient size and capacity so that the pressure in the dehydrating system can be quickly brought to the range of 2.5 to 1.0 mm. Hg. At this pressure, freezing will almost immediately take place and drying from the frozen state will proceed. The sudden freezing of course presupposes that materials containing dissolved gases have already been de-gassed as explained under "De-gassing Procedures".

There again attention is directed to the decided advantages secured through the present invention both as to improvement in the vacuum requirement and in the greater efficiency of the new "Cryochem" process over those heretofore in use. As indicated above, pressures on the order of 2.5 to 1.0 mm. Hg. or 2500 to 1000 microns are ordinarily used in the apparatus of the present invention, although not at all satisfactory for devices using low temperature condensers. In the "Cryochem" process, the critical pressure point is on the order of 2000-2500 microns and a very satisfactory rate of drying is obtained at 600-700 microns. In the processes heretofore available, such as the "Lyophile" which is limited to the use of low temperature condensers, the critical pressure point is at 700 microns. If the air pressure within the system is above this point (700 microns), the operation proceeds insufficiently fast to maintain the material in the frozen state even though it originally was frozen to a low temperature, and pressures [73] below 250 microns are necessary in order to get a fair rate of drying efficiency. Losses in pressure through leaks in the multiplicity of connections and the joints in any device of this kind must be carefully guarded against but cannot be entirely eliminated. A lower pressure is therefore used as a matter of safety and this has been found to be between 300 and 400 microns for the "Cryochem" apparatus while "Lyophile" apparatus require between 10 to 50 microns.

The above figures, comparing vacuum requirements for the system heretofore used with the present invention, indicate that the rate of fixation of the water vapor by the "Drierite" (calcium sulfate) is so much greater than that of the low temperature condenser that the mean-free path of the water vapor molecules can be much shorter without preventing maintenance of the frozen state by evaporation. The greater rate of fixation of water vapor is due to the much greater effective surface of the calcium sulfate particles as compared with the low temperature condensers. Even the interior of the porous particles of calcium sulfate react with the water vapor. This also accounts for the greater success obtained with "Drierite" as the desiccant in the self-freezing procedure. The final equilibrium vapor pressure is no lower; the rate of attainment is much more rapid than with low temperature condensation.

The *manifolds* consist of tubes of a size proportioned to take care of all the vapors from a plurality of outlets without offering resistance to the vapor-flow. Any number of manifolds may be attached around the chamber 21, depending on the size of the chamber and the amount of vapors the desiccant within it can efficiently absorb. Only two manifolds, 58 and 59, are shown, each with twenty-five outlets 52, placed in two rows at an angle of about 45° to [74] the horizontal as shown in Fig. 3. One end of tube 58 is screwed directly into the side wall 34 of chamber 21. An alternate scheme of attaching the manifolds is shown for tube 59 which is held in place by means of companion flanges 50. The outer ends of tubes 58 and 59 may be closed vacuum-tight in any convenient manner as, for example, with stoppers 57.

The outlets or container-connectors may be any suitable number consisting of simple tubes as at 82, Fig. 3, attached vacuum-tight at 81 to the main tube 58, and having provision on the outer end to receive the rubber tubing 83, holding the glass connector 84 in place. The elaborate and expensive quick-acting type of outlets heretofore necessary in the "Lyophile" apparatus are no longer needed in the "Cryochem" apparatus; because of the efficient action obtained through the use of the desiccant, the freezing procedure is materially changed.

We have, however, found that the type of outlet shown at 81 does not fill the needs of all conditions. In order to make the apparatus universal, the outlets should be so arranged that each individual glass container can be sealed-off separately in an aseptic condition and/or without influencing the adjacent containers or the apparatus as a whole. To provide for this desirable feature, the novel construction of a *separate vacuum-tight valve* 52 is provided in each outlet. One side of the valve 52 is attached vacuum-tight to the manifold tube 58 and the other side is provided with a suitable nipple 86 to receive the rubber tubing 87 holding the glass connector 51 from container 53 in place. Nipple 86 may, in some cases, be replaced by a tapered tube 97, Fig. 14, large enough to receive the rubber stopper 98. As shown in Fig. 3, the outlets are attached in an inclined position with the container-attaching and [75] pointing upward. The angle may be anywhere from 15 to 90° with the horizontal. The purpose of this is to guard against any possibility of contamination either during the attaching of the containers, during the drying cycle, or the sealing-off period. It also makes it more convenient in placing the containers in a horizontal position. The manifolds with their permanent outlets may not be sterile but, through the construction indicated, no bacteria or other micro-organism can fall into the container by gravitational forces. The containers as well as the glass and rubber connectors are, of course, sterilized before connection and all through the drying period the currents of air and vapors tend to carry any existing contaminants away from the product. The inclined position of the outlets is therefore of main importance while attaching the sterile containers and after closing valve 52 for the sealing-off operation. The importance of valve 52 in the individual outlets will be more specifically pointed out and described in connection with the sealing-off process. It may briefly be mentioned here that the novel combination of individual valves for each outlet of the manifold makes it possible to use flint or other inexpensive glass which does not have a particularly low coefficient of thermal expansion, such as is possessed by the very much more expensive "Pyrex" variety, and still seal-off the product in an aseptically unchanged condition. Heretofore only "Pyrex" glass could be used for the process of sealing-off under vacuo because the diameters needed for proper drying were too large to permit the proper sealing of ordinary glass tubes. The economy and efficiency resulting from the use of an all-flint glass container has extended the usefulness of the process into fields where the cost of "Pyrex" heretofore made it commercially impossible.

[76] The *container trays* 54, best seen in Fig. 3, used to support the containers during dehydration, are long rectangular basins (length to suit manifold and width to suit containers) supported by brackets 55 clamped to the manifold 58. Adjustable arms 56 may be used to extend out under the bottom 92 of trays 54. Additional adjustable brackets 95 and 96 with cross-rods 93 and 94 may be provided on arms 56 to suit special conditions. Trays 54 may be made from any suitable material, metal, hard rubber, porcelain, etc., but preferably from a non-rusting type. The bottom 92 may be flat or corrugated as shown at 90 and a wire screen 91 placed in the tray for the containers to rest on so as to keep them slightly above the bottom for free circulation of air all around as well as to permit any condensate forming on the containers to drain off.

As freezing mixtures for the rapid chilling of the containers are only required in some very special cases in the practice of the "Cryochem" process, no insulation of the trays is needed, resulting in a considerable economy as well as convenience. Nor is there any need for draincocks and other contraptions heretofore needed in similar devices.

The air-heating and circulating device 24, Fig. 2, may consist of a heater chamber 63, heavily insulated as at 64, with one end connected at 62 through valve 60 and duct 41 to the bottom outlet 40 of the desiccant chamber 21. At the opposite end 68 is connected a fan 69 or similar device which produces a rapid current of air directed to pass through the heater chamber 63 and around heaters 65 placed within it. For the purpose of illustration, we have shown electric heating elements 65 connected to a source of current supply at 66, but any other heat-supplying means such as gas, steam, etc., may be used.

To regulate the temperature of the air passing into [77] the desiccant chamber 21 through opening 40 is very important. It is evident that the bottom layers of desiccant are closest to the source of heat and would become over-heated and destroyed before the top layers had reached the needed temperature for regeneration. The control of the temperature is obtained through the novel arrangement of controlling the volume of air passing over the heat-supplying means. This is accomplished by adjusting intake 70 on fan 69 and also by setting the damper 67. The temperature may vary from 150°C., to 250°C., but for efficient regeneration it should be maintained from 175-200°C. For the purpose of checking the temperature of the air passing into the chamber 21, a thermometer 61 is provided in the connection at 62. For rapid regeneration, it is desirable to have a large volume of fast moving air at the proper temperature. Air under pressure might be used if available.

### PROPORTION OF CONTAINERS AND CONNECTORS:

In order to assure a large surface for the evaporation, the containers should be of a size and shape adapted to hold the material to be dried in such a position that a favorable surface-volume ratio exists and a maximum surface area is exposed to aid in efficient dehydration. They should be of convenient shape to handle when sterilizing, filling, freezing, sealing and storing. In addition, they should include provision for the easy introduction of the restoring liquid. In general, they must be of at least twice the capacity of the amount to be preserved. Long cylindrical vials have been found most efficient. The all-glass container, Fig. 18, affords the maximum safe protection but for some products the combination glass and rubber has been found satisfactory as well as desirable because of the somewhat greater ease of restoring the content to liquid form and withdrawing it aseptically. Heretofore, in order to avoid using the costly "Pyrex" sealing tubes,

[78] the rubber stopper was provided with an integral rubber sealing tube and special devices resorted to in sealing. This invention, however, eliminates any further need for either "Pyrex" or rubber sealing tubes and provides an all-glass container as shown in Fig. 18, where the cylindrical body 108 of the container-vessel is formed with an integral sealing tube 136. In this connection, it should also be noted that the use of such an all-glass container made from ordinary flint-glass has been made possible only through the new and novel method of sealing herein disclosed. The diameter of the exhaust tube 136, Fig. 18, must be of large bore, at least 4 mm. in diameter for containers to hold serum up to 25 ml.; and 7 mm. for quantities up to 100 ml. In diameters as large as those given above, only tubes of "Pyrex" glass will withstand the sealing under vacuo but, through the arrangement of parts and this new process, ordinary flint-glass tubes are successfully sealed under aseptic conditions.

CONTAINERS—FOR GENERAL USE: For optimum results in the practice of the "Cryochem" process, care must be exercised in the selection of the container used. While the same containers may be used as were described by Earl W. Flosdorf in connection with the "Lyophile" process, new and improved types are a part of this invention as well as a new and novel method of sealing the containers. This new sealing method overcomes the serious handicap of the "Lyophile" process where, when all-glass containers were required, these containers of necessity had to be made from the expensive "Pyrex" glass. The length of the storage period desired for the product in many cases determines the type of container that should be used. Containers with rubber stoppers such as are shown in Fig. 22, [79] or in Figs. 19 and 20, are quite satisfactory for relatively more stable products and will keep such products in perfect condition for a year or more while with very labile substances, such as a virus influenza, the combination rubber and glass containers could only be depended on for a maximum safe storing period of less than a month. For long periods, the diffusion of moisture and air through the extensive area of thin rubber in stoppers of the type shown in Figs. 24 and 25 is too great, particularly if the temperature or relative humidity, or both, are high. Heretofore, even when rubber stoppers were satisfactory, it was necessary, unless they were provided with the somewhat unsatisfactory integral rubber exhaust tubes, to use sealing tubes of "Pyrex" glass for sealing under the original vacuum.

The limitation to the use of "Pyrex" has now been overcome by the type of containers and method of sealing developed as part of this invention. When rubber stoppers are desirable, the type of container shown in Figs. 19 and 20 may be used. It should be noted that the container takes the form of a relatively long cylinder which is reduced at one end into an ordinary bottle neck style of suitable size to receive certain types of rubber stoppers while the other end is drawn down into a tube of suitable diameter for efficient exhausting and of a length convenient to assemble on the apparatus and providing space for constricting and sealing. With the container shown in Fig. 20, the vaccine rubber stopper shown in Fig. 24 is used. The stopper body 133 fits tightly into the neck 141 of the container. The recess 134, corresponding with a dimple in the outside surface of the stopper, provides a thin membrane to make it possible for the user to pierce through with the syringe needle when adding water to restore the dry content in preparing it for withdrawal and use. The small [80] diameter exhaust tube 143 is, after the container is filled, attached to the apparatus as may be seen in Fig. 3, at 88 and 89, or as shown step-by-step in Figs. 8, 9 and 10. A line 144 is shown across the tube 143, indicating the point where the tube may be reduced in diameter as shown in Fig. 11 at 111 by means of a flame 112, so that the process of sealing under vacuum may be performed as shown in Fig. 12 at 114. The container shown in Fig. 19 is of a slightly different shape. It has the long cylindrical body 138, but the neck 137 is considerably reduced so as to take the hooded vaccine stopper shown in Fig. 25, the body 131 fitting into the neck 137 while the hood 130 is pulled down over the outside of the neck 137 to provide additional protection against the diffusion of moisture and air through the joint. The stopper is provided with the same recess 132 corresponding with a dimple in the outside face as was pointed out in the stopper shown in Fig. 24. The body 138 is drawn down into a small diameter tube 139 which, after filling, is connected to the apparatus as previously described and shown in Fig. 3. A line 140 across the tube indicates where this may be reduced in diameter as shown in Fig. 11 and subsequently sealed off as shown in Fig. 12.

An all-glass type of container is shown in Fig. 18. This has the long cylindrical body 108 closed at one end and at the other end the exhaust tube 136 in proportion to suit its size. The line 135 across the exhaust tube 136 indicates the probable point where the tube may be reduced or constricted in diameter and subsequently sealed as indicated in Figs. 11 and 12. Through the novel process of turning off the manifold valve 52, at the completion of the dehydration period and admitting dry sterile air by means of the air dryer and sterilizer, Fig. 13, and then constricting [81] the exhaust tube diameter, the feasibility of using ordinary flint-glass in the "all-glass" container, in place of the more expensive "Pyrex" glass and still be able to seal under vacuum, has been made possible.

Ordinary flint-glass test-tubes may be used successfully in dehydrating cultures and viruses. An example of such a tube is shown in Fig. 21. The amount of material to be dried is so very small, usually only about 0.05 ml., that it can easily be confined in a tube of this size, about 7 mm. outside diameter by about 95 mm. in length. The test tube, in Fig. 21, is shown with a straight body 128, while the same tube is shown in Fig. 14 at 101 with a section 129 constricted so that it may be sealed aseptically and under the original vacuum. A slightly modified tube to hold a somewhat greater quantity of material is shown at 102 of Fig. 14. On this tube, however, the connecting tube 103 has been constricted at 129 so that it also may be sealed off while under the original vacuum. These small "test-tube-containers", Fig. 14, hold so little material that efficient dehydration of the content is possible even though they are constricted before attaching to the apparatus. They may, of course, be used during desiccation in their standard size and then constricted after the completion of the dehydration period in the same manner as the larger containers, but before attaching they are more convenient to handle and therefore some time may be saved if they are constricted before use.

Regardless of the size or type of receptacle used, the exhaust tube ought to be reduced in diameter to between 1 and 2 mm., for a sufficient distance to provide ample room for the final sealing after the secondary vacuum has been drawn.

[82] The "S" type of tube is shown in Fig. 15. Both ends of the body 51 are bent at suitable angles so that, when the tube end 124 is connected as shown in Fig. 3 by means of a rubber tubing 87 to the manifold outlet 86, the other end 125 will make a horizontal connection to the container through another rubber tube 88. A glass-connector-tube of this shape is very desirable for all general-purpose desiccation. The tube should be rigid but convenient to sterilize, which is necessary before each use. Such a tube in sterilized condition is shown in Fig. 8, ready for assembling with container and manifold.

For use in connection with the small test-tube-container, double or triple outlet "L"-tubes are provided as shown in Figs. 16 and 17. The connection for a double "L"-tube is shown at Fig. 14 where the body part 99 is pressed into a stopper of the type shown in Fig. 23 and the rubber stopper then pushed into a suitable cavity 97 of the manifold valve 52. The double tube shown in Fig. 16 has two side outlets 122 and 123 while the triple tube shown in Fig. 17 has two central side outlets 121 in addition to the end side outlet 120. The method for use of these multiple outlet connector tubes is shown in Fig. 14, where two test-tube size containers are connected by means of the rubber tubes 100 to a double "L"-tube 99. The use of these multiple outlet connector tubes of course represents a tremendous saving in time because, the quantities being so small in the test-tube, a great number of them can be desiccated at one and the same time through these combinations.

ASSEMBLING AND CONNECTING TO APPARATUS: As mentioned above, all parts should be so constructed that they may be readily sterilized and kept sterile for a reasonable length of time. The "S" connector-tube shown in Fig. 8 [83] represents a sterilized tube 51 assembled with a sterilized rubber tube 88. To maintain the interior of the tubes in a sterile condition, cotton plugs 104 and 105 are quickly inserted and left there until the connector is assembled with the other parts. In Fig. 8*a* is shown the ampoule type of container described under Fig. 18 but now in a sterile condition and provided with the cotton plug 106 to maintain sterility of the interior surfaces. In Fig. 9, the container 108 of Fig. 8*a* has been filled with its material 109 and assembled to the connector tube 51 through the rubber tube 88. The cotton plug 104 is still remaining in the glass connector so as to maintain sterility of the interior surfaces. It should be kept in mind that a plurality of containers must be handled at one and the same time, usually reaching in number from 25 to 50 or more. It is therefore important that, while completing the filling of such a large number of containers, they all may be retained in a sterile condition so that an aseptic product may be obtained. When ready to assemble the filled containers to the manifold of the apparatus, the cotton plug 104 as shown in Fig. 9 is removed and the connector-tube 51 assembled by means of rubber tubing

87 to the hose-stem 86 of manifold valve 52. While making this connection, the valve 52 probably would be in the closed position as indicated in Fig. 11 and remains in the closed position until all of the containers have been connected. That, however, is not necessary with the present invention. Each manifold outlet being provided with a separate valve, the dehydration may commence instantly that one of the containers has been filled and connected to the manifold. It is evident that, during this connecting period, all the manifold valves would be closed until the container has been connected at which time the valve may [84] be instantly opened and the dehydration commenced on that particular container.

DEHYDRATION PROCEDURE: Three methods of procedure are in use: (1) PREFREEZING of the material in the container before starting to desiccate; (2) DEGASSING-SELF-FREEZING accomplished by controlling the vacuum during the early stage of the desiccating period; and (3) SELF-FREEZING DEHYDRATING.

PREFREEZING—With the use of the "Cryochem" apparatus, the low temperature freezing mixtures of the "Lyophile" process are no longer necessary. A temperature of minus 5° to minus 15°C. is usually quite satisfactory. This can easily be produced by a salt-ice mixture spread over and around the containers on the tray 54, or the containers may be chilled and the material prefrozen by resorting to mechanical refrigeration. Dry-ice of course may be used but the resulting low temperature of minus 50°C. is unnecessary. With the material in the frozen state and the containers 63 properly attached, as described above, to the manifold valve 52, the evacuation is carried out to the limit of the capacity of the pump 22. For extremely labile substances, such as certain viruses, there is evidence that these deteriorate somewhat during "Lyophile" processing and it is therefore of advantage in the "Cryochem" process to maintain a temperature of about —10°C., for such products until the desiccation is as complete as is possible to obtain at the aqueous tension differential produced between minus 10°C. and that of the "Drierite". The temperature is then allowed to rise very gradually to that of the room at such a rate that the temperature at any time is never much above that necessary to produce a small further differential in aqueous tension. In this fashion, the temperature of the product is always at a minimum for the amount of residual water still remaining during the final stages of desiccation and deterioration during processing is kept to a minimum.

[85] DEGASSING SELF FREEZING—If solutions containing protein and/or dissolved gas are placed under high vacuum without first being reduced to the frozen state, they immediately froth and the material in the small individual serum-containers is pushed out through the exhaust tube. Self-freezing of such products in the final containers has therefore not heretofore been feasible. Through this invention, this handicap is overcome by introducing the preliminary step of degassing. After the filled containers have all been connected to the manifold as shown in Fig. 3, the vacuum pump 22 is started but, instead of permitting the pump to operate at its full capacity, it is throttled down so as to keep the pressure at a point where frothing just begins to occur. The throttling down of the pump is accomplished by means of valves 37 and 38, Fig. 1. Valve 38 may be partially opened, thus providing a very effective leak for the pump to take care of or valve 37 may be partly closed, or both valves may be so operated. The system should be kept at this pressure for one quarter to one hour, when sufficient gases will be removed so that de-gassing may be accelerated by further lowering the pressure. As an aid in speeding up the de-gassing period as well as to guard against the loss of material through the exhaust tubes, the freezing trays may be tilted to an angle of about 45° so that the material in the containers will settle in the

bottom of the container instead of along the horizontal side as is desirable during the self-freezing period. All through the de-gassing period, there is a slight evaporation of moisture which is sufficient to keep the material in a cooled condition. The time required for the de-gassing period varies with different materials. The de-gassing step should be stopped as soon as permissible so that the self-freezing may be brought about at as early a time as [86] possible by allowing the pump to evacuate to the limit of its capacity. At this point, of course, valve 38 should be closed vacuum-tight and valve 37 fully opened. As soon as the pressure falls to the range of 2.5 to 1.0 mm. Hg., freezing will almost immediately take place and drying from the frozen state proceeds. Although the pressure now is well below the point where boiling ordinarily should occur for the given temperature of the product, this does not happen because the dissolved gases have been removed. Usually the temperature of the substance falls for a time a few degrees below its freezing point so that it is simultaneously super-heated and super-cooled. Freezing will then set in with the suddenness characteristic of that encountered with super-cooled solutions not unlike the sudden crystallization of a super-saturated solution.

In this manner, the advantages of rapid freezing are obtained without the use of refrigerants. Upon the initial freezing, the temperature of course rises to the freezing point but, when freezing is complete and as evaporation proceeds, the temperature falls to the range of minus 10° to minus 20°C. From then on the temperature slowly rises until the dehydration is complete and the container and the material are at room temperature.

SELF-FREEZING-DEHYDRATION—The consistency of certain products permits the practice of this most convenient and economical process. All that is required is to connect the filled sterile containers, start the desiccation and let this proceed for the time required to obtain the desired dryness.

[87] THE PRODUCT OF THE DEGASSING-SELF-FREEZING PROCESS—Inasmuch as there is a slight degree of desiccation from the liquid state, the final appearance of the product is one of a highly porous mass covered with a glossy amorphous film. The product dissolves with remarkable speed and completeness; in fact, with certain products, there is some indication that the solubility-characteristics may be superior to those produced when other means of preliminary freezing are used.

TIME REQUIRED FOR DEHYDRATION—Under some conditions, the time required for the completion of desiccation by the present invention is reduced to one-half or one-third of that required by devices heretofore available. The time required varies with the thickness of the layer of the material in the container and with the nature of the material itself. Usually it is safe to allow half again as much time as is required for the temperature of the containers to reach that of the room. Because of varying conditions and the many different substances that are successfully dehydrated by this process, it is preferable to allow the determination of residual moisture-content to decide the duration of processing. The purpose for which the product is preserved should naturally also be taken into consideration when deciding on the permissible residual moisture-content.

The residual moisture-content of serum-like substances (calculated in terms of the weight of the dry solids) may be reduced to below 2% by the use of the present invention. If sufficient processing time is allowed, the final moisture-content can be reduced to less than 0.1% which corresponds to 99.99% removal of the original water. The time may therefore be different for every product and may vary from 10 to 25 or 30 hours as determined by the final dryness desired for the product.

[88] AIR-DRYER AND STERILIZER: In order to seal-off the dehydrated material in its container in an aseptic condition, a new and novel "dry-air-sterilizer" (Fig. 13) is required.

This may consist of an ordinary syringe barrel containing a properly confined chemical desiccant and a standard Luer needle attached to it. In Fig. 13, the barrel, which may be of cylindrical or any other convenient shape and of suitable length, is shown at 115. One end of the barrel may be opened or provided with a removable or hinged cover (not shown), while the other end is reduced into a hollow needle 110 so that the rubber connectors 88 may be easily pierced for the admittance of dry sterile air. The reduced portion 110*a* may be formed into a neck provided with suitable means for the attachment of a syringe needle. In the bottom of the barrel occupying about one-quarter of the space is placed a bacterial filter such as sterile cotton 116. One-third of the remaining space is filled with a regenerable chemical desiccant 117 and on top of this is placed another cotton plug 116, occupying about half the remaining space. The air dryer sterilizer is prepared by heating in an ordinary oven for a half-hour at a temperature of 200° C. "Drierite" is preferred as the desiccant in the barrel and this being a regenerable material the device, of course, can be used an indefinite number of times because the re-sterilizing process, at the same time, regenerates the desiccant. To prevent contamination or the loss of effectiveness of the desiccant, stoppers (not shown) may be used at both ends, thus keeping the instrument ready for immediate use.

The air-dryer-sterilizer is a simple device but, without it and its proper use, the sealing of the dehydrated product into the protecting receptacle in an aseptic condition would [89] be impossible. The invention of this device, therefore, is of utmost importance in the successful practice of the process herein described. Although it may be considered to be an instrument detached from the rest of the apparatus, it is definitely a necessary part of it and indispensable in the practice of the one most important method of sealing herein described.

Sealing Procedure: The method used and the care taken in sealing the containers depends, of course, upon the type of product being processed. Through the practice of this invention, three methods are available and have proved very satisfactory:

(1) Aseptic: When the product must be preserved in an aseptic condition, the procedure to follow is to shut the individual manifold valves 52 (Figs. 3 and 11), admit dry, sterile air to an evacuated container 108 through the use of the air-dryer-sterilizer shown in Fig. 13 by piercing the rubber-tube-connector 88 with the syringe needle 108 as shown in Fig. 11. After the dry sterile air has been admitted, the exhaust tube 107 can be constricted at 111 for a distance of 10 to 15 mm., to a diameter of about 1-2 mm. by means of a gas-flame 112. As soon as constricting is finished, valve 52 is again opened and re-evacuation of container 108 proceeds. The same procedure is carried through, one by one, on the remaining containers, the pump 22 in the meantime being in continuous operation to keep the system at a low pressure and in aseptic condition. After a few minutes of evacuation, container 108 is ready for the sealing operation and is sealed, Fig. 12, by applying a gas-flame 112 at section 114, previously prepared by constricting. While sealing-off, the container 108 is severed from the assembly and we have the finished product as shown in Fig. 12*a*. The particular form or shape of container as shown and described in this connection is only one of sev-
[90] eral types now in use and referred to here as being a preferred type, but we do not limit ourselves to the particular shape nor to size and proportion specified.

The finished product is an "all-glass" receptacle, Fig. 12*a*, containing a dry (99.9%) very porous substance 113, with properties as described above, occupying approximately the same space in the container 108 as the original liquid, imperviously sealed aseptically and in vacuo for storage for an indefinite length of time at any convenient temperature. At the time of use of the product, sterile, distilled water is admitted from a syringe through the exhaust tube 107 which should be previously prepared by marking a file scratch at 107*a* so that the sealed portion can easily be broken off. The dry material readily dissolves and is quickly restored to its original consistency or to the consistency desired.

Containers such as those shown in Figs. 14 and 21 are treated in exactly the same manner as described above for all-glass containers.

Containers such as those shown in Figs. 19 and 20, which are provided with a vaccine stopper, are sealed aseptically in the same manner as that described above for the all-glass container. The preserved material, however, within the receptacle is restored by admitting sterile distilled water by means of a syringe, the needle being pushed through the thin membrane indicated at 132 in Fig. 25 and at 134 in Fig. 24, the vacuum within materially aiding in dissolving the material.

(2) Non-Aseptic: All products do not require to be sealed in a perfectly aseptic condition. At the end of the dehydration period on such products, the pump 22 may be shut down and valve 37 (Fig. 1) closed. Air may then be slowly admitted into the system by opening valve [91] 38. The air so admitted becomes dry on its way through the desiccant chamber 21 before reaching manifold 58 and the container 53. When atmospheric pressure has been restored within the desiccating system, the manifold valve 52, Fig. 3, may be closed and the containers 53, one by one, removed from the manifold connection and the exhaust tube 107, Fig. 11, constricted as indicated at 111. Each container 108 with its now constricted exhaust tube 111 may again be connected to the manifold but we have found it more convenient to attach the containers directly to a vacuum pump for the purpose of producing a secondary vacuum in the container before sealing, which is accomplished in a manner similar to that shown at 114, Fig. 12. Connecting directly to a vacuum pump is for the purpose of affecting rapid evacuation. Evidence indicates that, for ordinary biologics, a vacuum of 1 mm. to 0.2 mm. is quite satisfactory. The

process of restoring such products is, of course, identical with that described for the aseptic product.

(3) NON VACUUM STORAGE: Sealing in vacuo is unnecessary for certain products, such as human milk; in any event, however, the container must be hermetically sealed in order to exclude moisture. Ordinary nursing-bottles of the type shown in Fig. 22 are suitable for such products. We have found that for a product of this kind and containers of the type referred to, the filling of one-quarter the capacity is the most efficient proportion, allowing for very rapid completion of desiccation. The containers are assembled to the sterile connectors of the manifold by placing a stopper of the type shown in Fig. 23 in the neck 119, Fig. 22. At the end of the desiccating period, sterile air is admitted, the processing stopper, Fig. 23, is removed and a stopper of standard vaccine type, Fig. 24, quickly introduced into the neck 119 with aseptic precautions.

[92] It is, of course, understood that, in cases where vacuum storage is not necessary, any one of the containers, whether it be the all-glass or the combination rubber-glass type, may be sealed off hermetically without re-evacuation after the air has been admitted either aseptically or otherwise. Likewise an inert gas such as nitrogen or argon may be admitted in place of the air.

GENERAL DEHYDRATION PROCEDURE: When used as a dehydrator, the valve 60, Fig. 1, is tightly closed. The manifold valves 52, Fig. 3, may if so desired all be closed and the valve 37 opened while the valve 38 is tightly closed so that evacuation may commence and the air within the voids of the desiccant 47 will be partially removed while the containers are connected to the manifold outlet. The containers are only filled to about ½ their capacity and attached to the manifold so that they rest on the screen 91 of the tray 54. When using the small containers shown in Fig. 21 for dehydrating micro-organisms, the arrangement shown in Fig. 14 is used. These containers being so small, the use of tray 54 is not necessary. When de-gassing, self-freezing is practiced, the trays are placed so as to support the containers 53 at an angle of about 45° but as soon as the de-gassing period is completed the trays 54 are returned to the horizontal position. For products requiring pre-freezing, any ordinary method of freezing may be employed such as water-ice and salt mixtures, dry ice or mechanical refrigeration. Evacuation is carried out to the limit of the capacity of the pump and the pressure preferably kept between 700 and 300 microns for the required time which may be anywhere from 10 to 30 hours or more. The construction of our apparatus is so simple that during this period it will require no attention excepting an occasional check-up to see that no leaks have opened or the vacuum-producing means [93] have failed. The apparatus described in this invention may be run for a period greatly in excess of that required for the dryness desired and no bad effects caused either to the product being processed or to the desiccant, or without additional energy costs except for the running of the vacuum pump.

When the desired dryness of the product is obtained or later when convenient, the containers 53 are sealed-off in any convenient manner or according to one of the three methods heretofore described. One of the resulting products obtained through the use of our invention is shown in Fig. 12*a*. This is the ampoule type "all-glass" container with the dehydrated material 113 sealed into the container 108 aseptically and under vacuum. We do not limit ourselves to the particular shape, sizes, combination glass and rubber container, etc., shown and described herein.

REGENERATION OF THE DESICCANT: When used as a regenerator, Fig. 2, valves 37 and 38 are closed and valve 60 opened to the air-heater-circulator 24. The lid or cover 26 shown in Fig. 1 is removed and replaced by a loose-fitting disk 27, Fig. 2, for the purpose of permitting the air to escape slowly as it rises between the desiccant baskets 46 or through the desiccant 44. The regenerating temperature required is between 150 to 250°C; 180° being preferred. The current of air should pass rapidly into the desiccant chamber and be maintained within the temperature range mentioned in order that rapid regeneration may be obtained. The air enters through duct 40 at the bottom of the desiccant chamber and, as the "Drierite" rises in temperature, the water which it contains is evolved. At the beginning of the regeneration cycle, the regeneration zone is in the lower portion of the chamber. As the supply of heat is continued, the regeneration zone gradually rises until it reaches the top of the chamber. Regardless of the type of desiccant basket used, thermometers are provided [94] as indicated at 43*a* so that the temperature can be checked from time to time. When the temperature indicated by a thermometer placed in thermometer well 43 or into the desiccant 47 of the top basket shown in Fig. 1 reaches 150°C., it is an indication that regeneration has been completed. At a given temperature, the time of regeneration is a function of air-circulation and layer-thickness. In four hours it will ordinarily be completed, but this should be checked under actual conditions and for that purpose we have provided the test plug 79, shown in Fig. 5, within the basket.

By removing this test plug from the desiccant chamber and making a moisture determination in the desiccant at various points, the condition of the desiccant may be determined. In the apparatus shown in Fig. 1, the desiccant 47 is conveniently accessible and the ordinary methods for moisture determination are used. Upon completion of the regenerating period, the "Drierite" should not contain more than 0.3% moisture. As soon as the regeneration is completed, the insulated disk 27 is removed and cover 26 shown in Fig. 1 loosely placed on top of the chamber 35 to prevent contamination. Naturally, the desiccant chamber 35 and the manifold 58 as well as other connecting parts all are sterilized during the regeneration period and, as soon as the desiccant has cooled down to room temperature, the apparatus is ready for use as a dehydrator.

Instead of using the open system as described above for the regeneration of the desiccant, we have found it more economical to use a closed system and recirculate the heated air. Such an arrangement is indicated by the duct 70*a* shown in dotted outline connecting a suitable opening in lid 28 with the intake 70. When this system is used, a few of the manifold valves 52 are left open to permit some of the hot air to escape and carry off the released water vapors.

[95] We do not intend to be limited save as the scope of the attached claims may require.

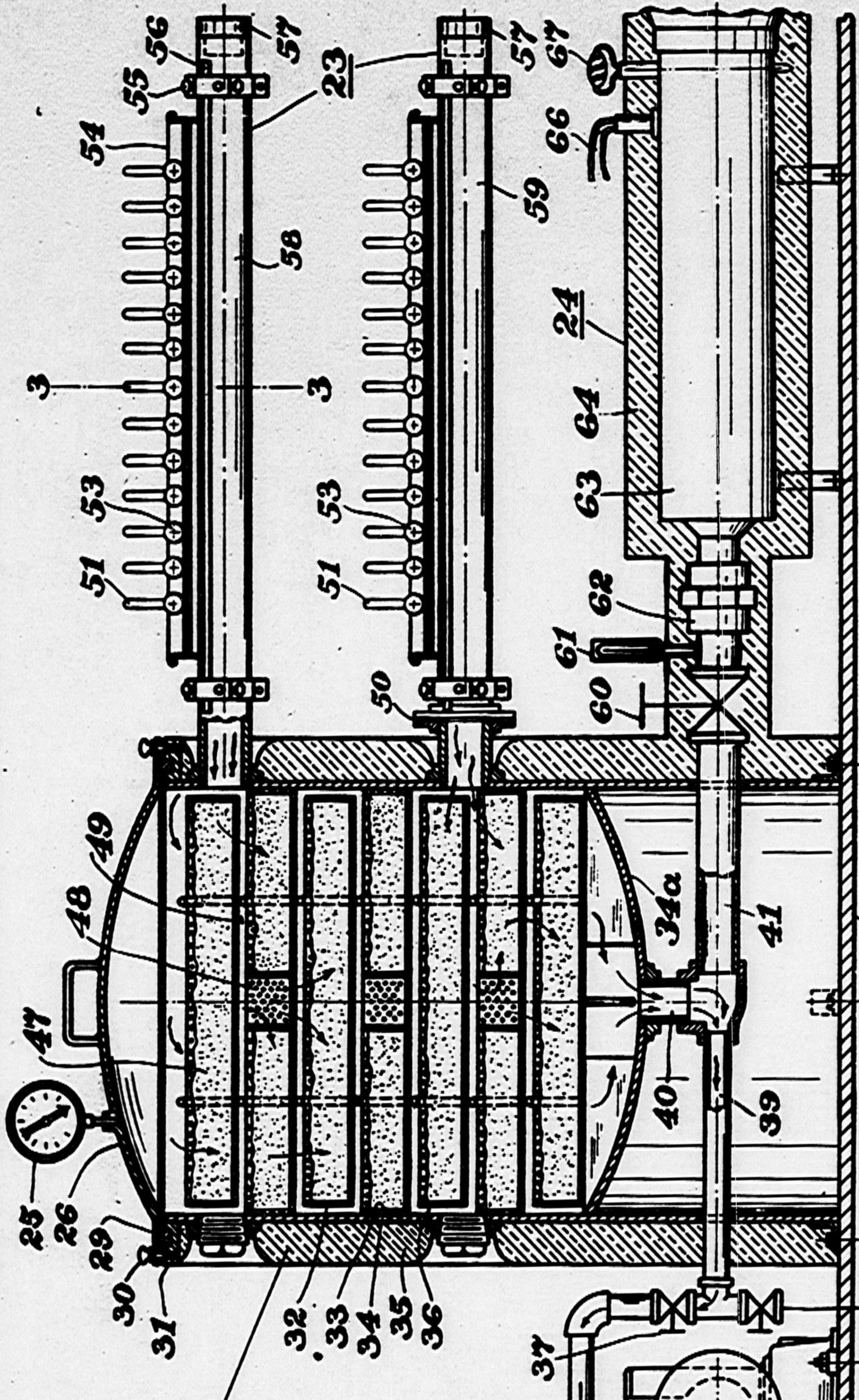

Fig.1.

INVENTORS
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ATTORNEY.

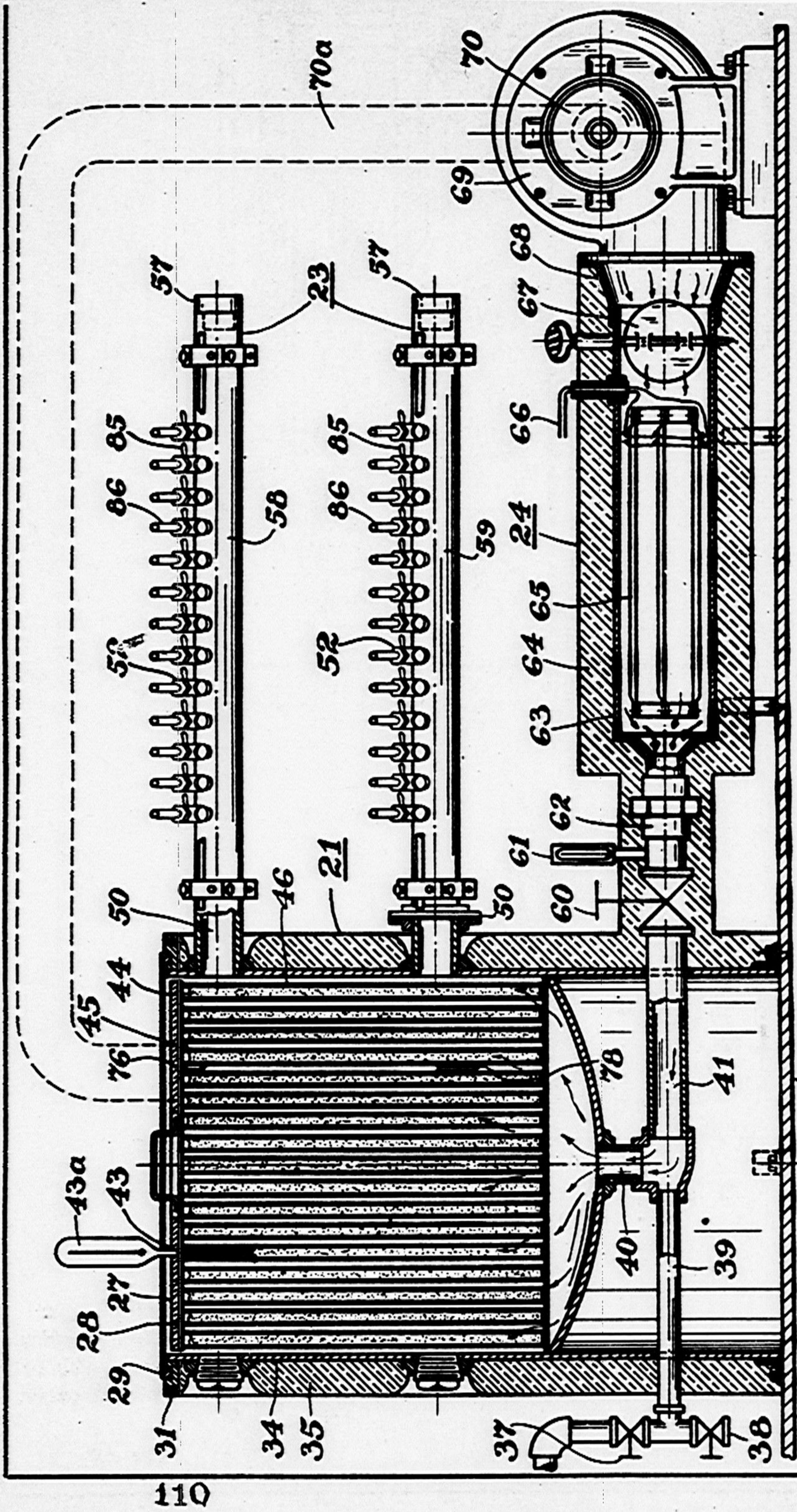

Fig. 2.

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F. Joseph Stokes, Jr.
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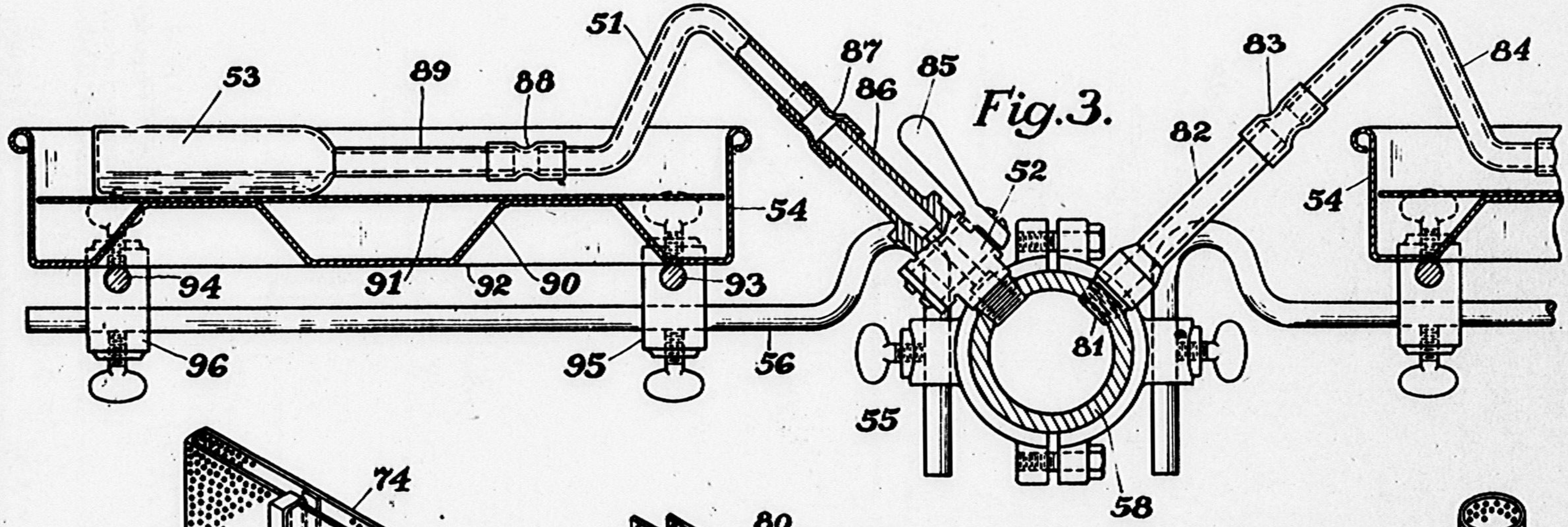

Fig. 3.

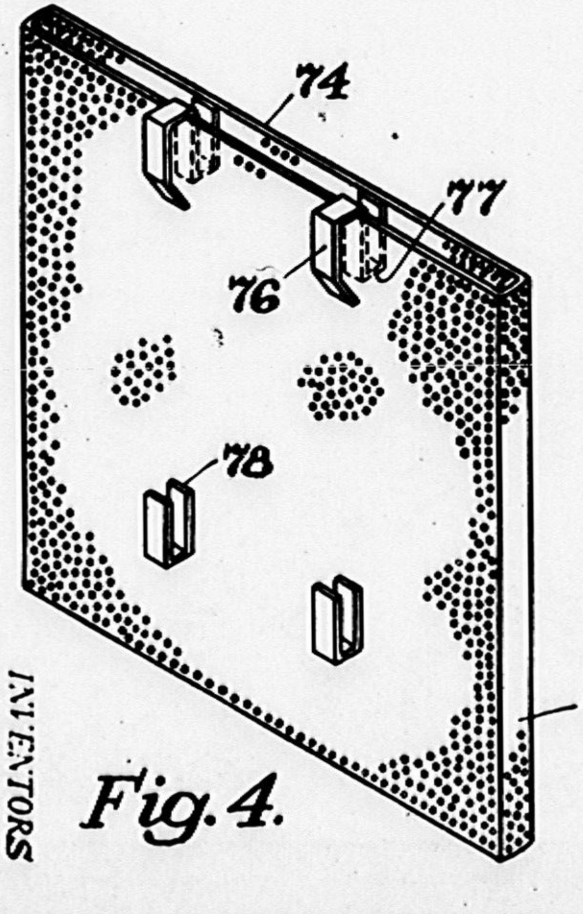

Fig. 4.

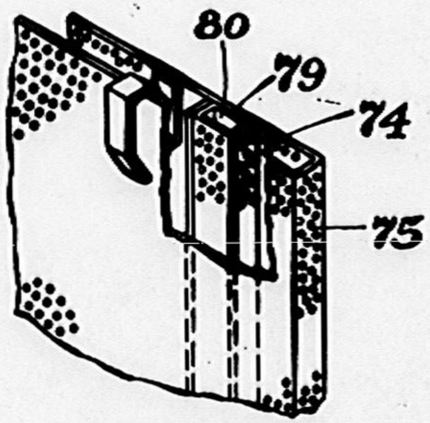

Fig. 5.

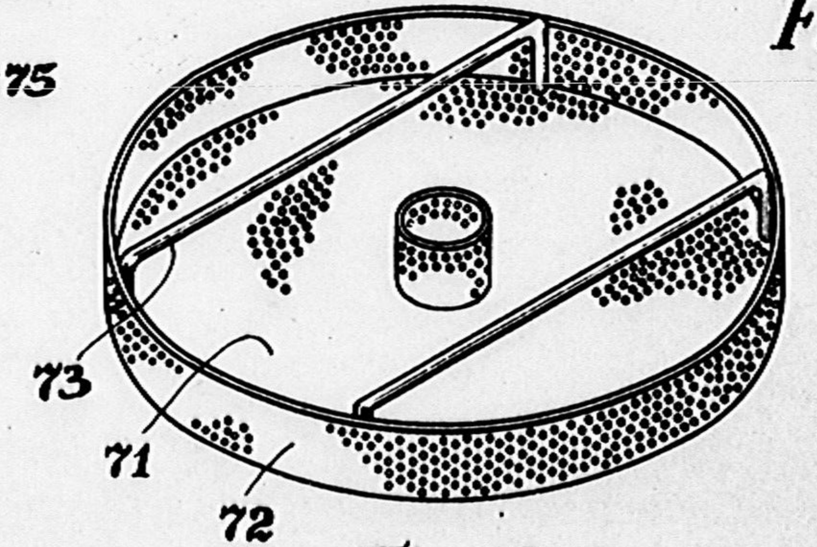

Fig. 6.

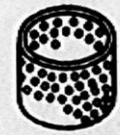

Fig. 7.

INVENTORS
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F. Joseph Stokes, Jr.

BY

ATTORNEY.

Fig.8.

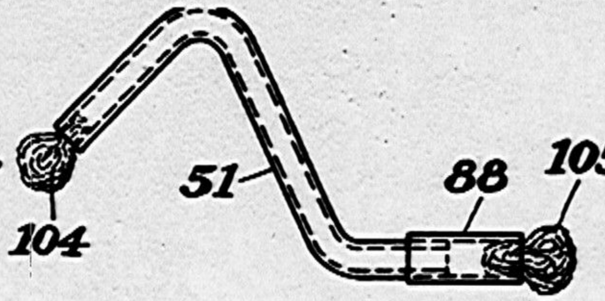


Fig.8a.

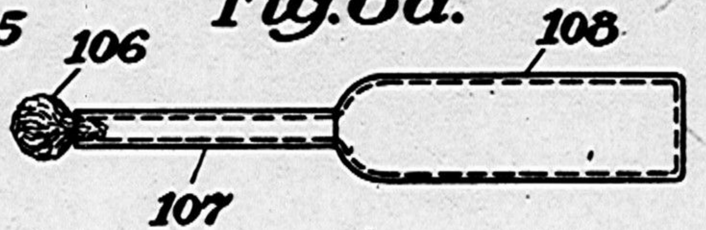
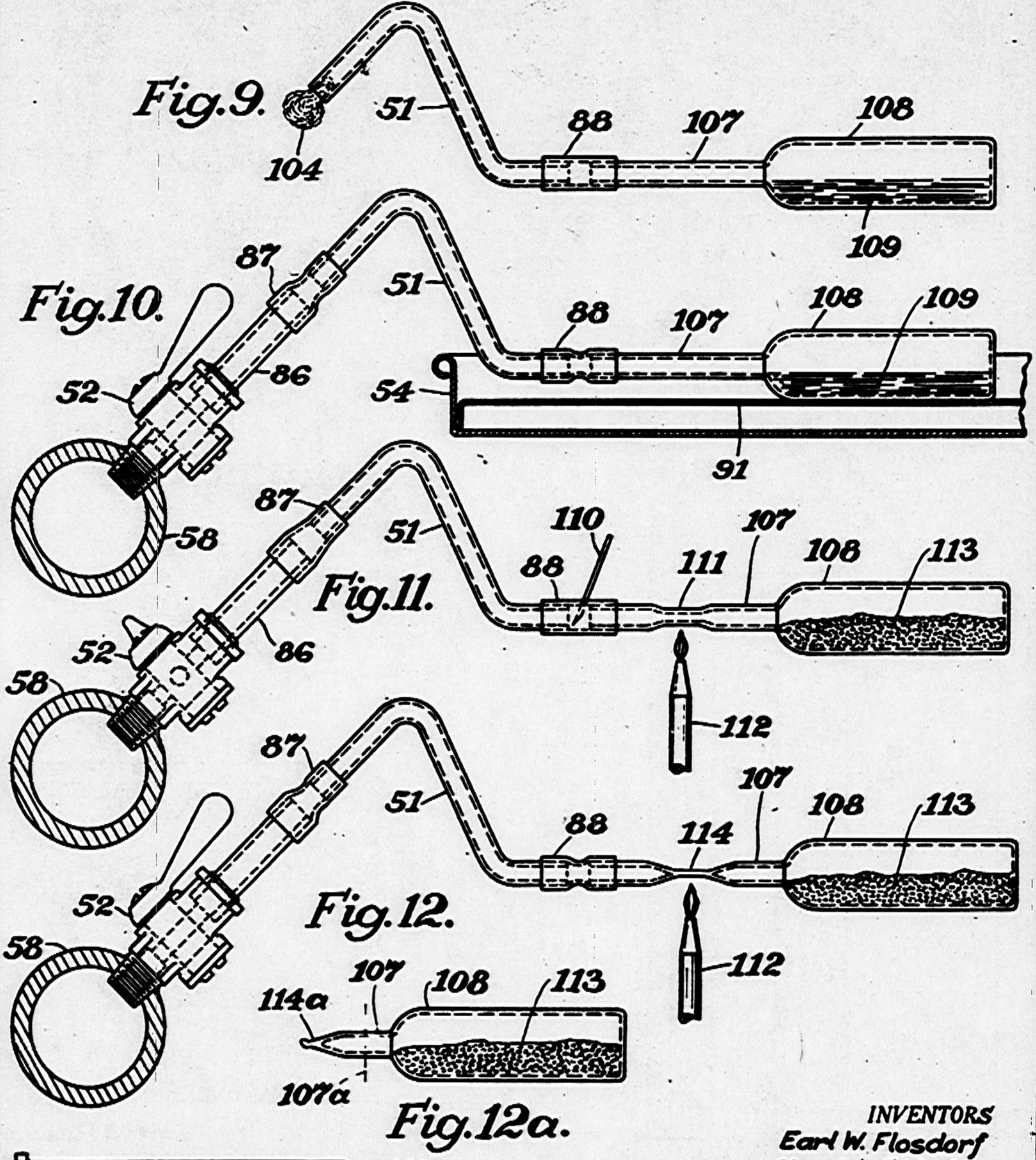


Fig.9.
Fig.10.
Fig.11.
Fig.12.
Fig.12a.



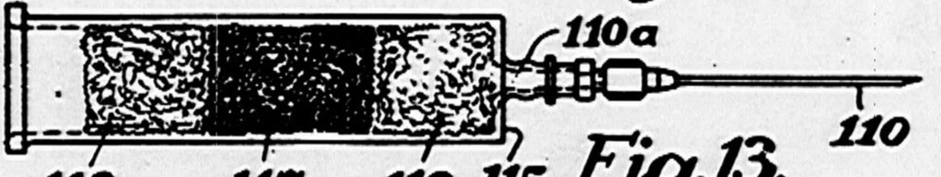


Fig.13.

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Charles I. Westin
F. Joseph Stokes, Jr.
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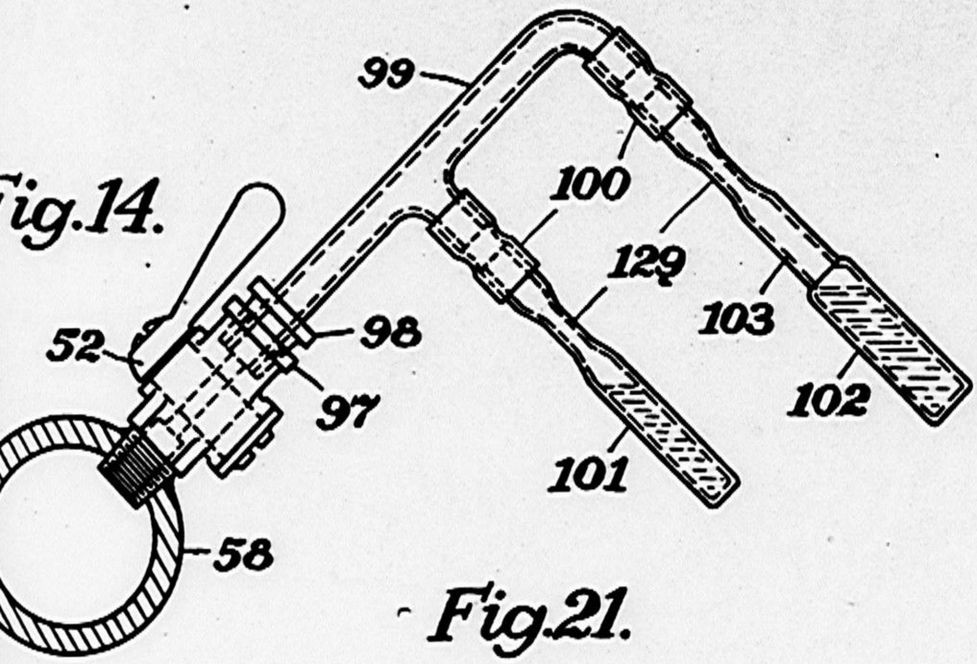


ig.14.

Fig.22.

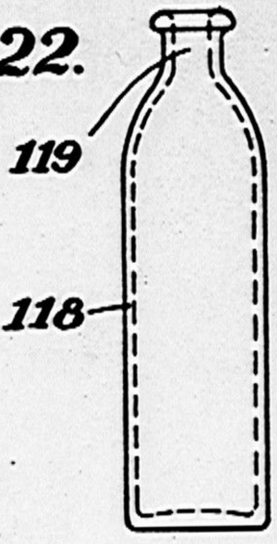


Fig.21.

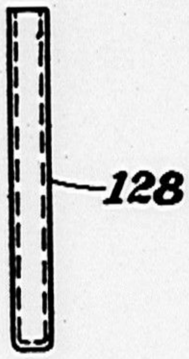


Fig.23.

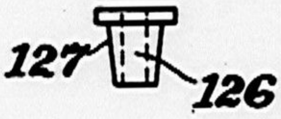


Fig.25.

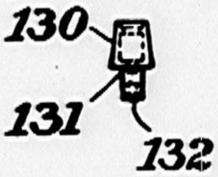


Fig.24.

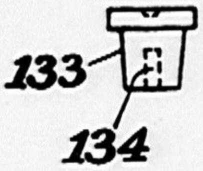


Fig.15.

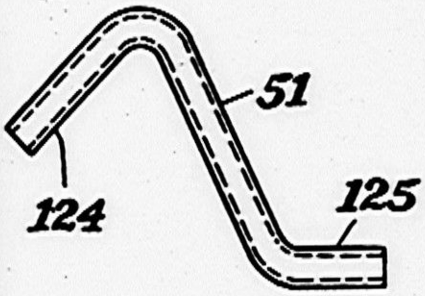


Fig.18.

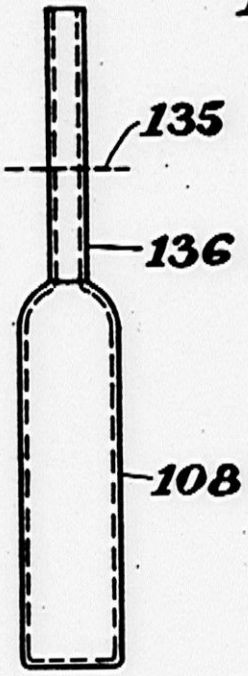


Fig.19.

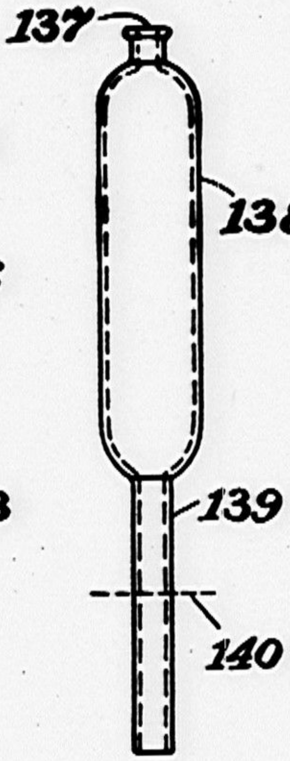


Fig.20.

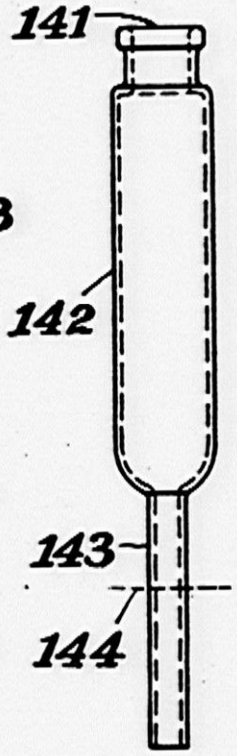


Fig.16.

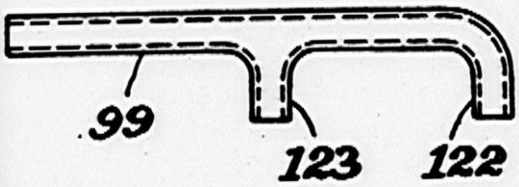
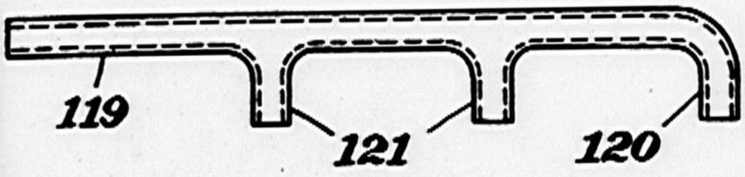


Fig.17.



INVENTORS
Earl W. Flosdorf
Charles J. Westin
F. Joseph Stokes, Jr.

BY

ATTORNEY.

## [179] PLAINTIFF'S EXHIBIT 5.

### List of Installations of Cryochem Apparatus.

| Name | Date sold |
|---|---|
| Veterinary Research Lab.<br>Kenya Africa<br>R. Daubold | 9/15/37 |
| Technopromimport<br>Moscow 9<br>Pr. Chudoshestw. teatra 2<br>Russia | 1/14/38 |
| Allied Laboratories, Inc.<br>(for Pitman Moore Biol.<br>Labs. Zionsville, Ind.) | 2/10/38 |
| Bryn Mawr Hospital<br>Bryn Mawr, Penna. | 2/23/38 |
| E. R. Squibb & Sons<br>New Brunswick, New Jersey | 3/16/38 |
| Children's Fund of Mich.<br>Detroit, Mich. | 3/21/38 |
| Georgetown Univ. Med. School<br>Washington, D. C. | 3/25/38 |
| Camden Health Dept. Lab.<br>Municipal Hosp. through<br>St. of N. J.—N. J. State | 4/1/38 |
| University of No. Carolina<br>Chapel Hill, N. C. | 4/4/38 |
| University of Michigan<br>University Hospital<br>Dept. of Pediatrics &<br>Infectious Diseases<br>Ann Arbor, Mich. | 4/8/38 |

*Plaintiff's Exhibit 5.*

[180]

| Name | Date |
|---|---|
| Harvard University<br>General Hospital<br>Boston, Mass. | 3/16/38 |
| Columbia University<br>for School of Tropical Medicine<br>Puerto Rico | 5/16/38 |
| Los Angeles County General Hospital<br>Los Angeles, Calif. | 5/18/38 |
| Henry Phipps Institute<br>Philadelphia, Pa. | 5/23/38 |
| University of Pennsylvania<br>Philadelphia, Penna.<br>(Maloney Clinic) | 6/9/38 |
| Cornell Univ. Med. College<br>New York City | 6/21/38 |
| University of the Phillipines<br>Manila, P. I. | 8/30/38 |
| Lederle Laboratories<br>Pearl River, New York | 10/4/38 |
| Connecticut State College<br>Animal Diseases Dept.<br>Storrs, Conn. | 10/19/38 |
| Haffkine Institute<br>Bombay, India | 10/31/38 |
| War Dept. Medical<br>Supply Officer<br>**Army Medical Center**<br>Washington, D. C. | 12/2/38 |
| Lederle Laboratories<br>Pearl River, New York | 12/9/38 |

*Plaintiff's Exhibit 5.*

[181]

| NAME | DATE SOLD |
|---|---|
| Gov't of Puerto Rico<br>Ordered through the U. S.<br>Dept. of the Interior<br>Insular Purchasing Agency<br>New York City | 12/21/38 |
| University of Hong Kong<br>Hong Kong, China | 12/23/38 |
| Kansas State Board of Health<br>Topeka, Kansas | 1/20/39 |
| Imperial Japanese Army<br>Through their N. Y. office | 1/25/39 |
| Buffalo General Hospital<br>Buffalo, New York | 1/31/39 |
| Michigan State Dept. of Health Labs.<br>Lansing, Mich. | 2/2/39 |
| University of Wisconsin<br>Madison, Wisconsin | 2/4/39 |
| St. Louis University<br>St. Louis, Mo. | 2/6/39 |
| Princeton University<br>Princeton, N. J. | 3/15/39 |
| Los Angeles County<br>General Hospital<br>Los Angeles, Calif. | 5/1/39 |
| National Institute of<br>Health<br>Washington, D. C. | 5/24/39 |
| [182] Medical Supply Office<br>Army Medical Center<br>Washington, D. C. | 5/26/39 |

*Plaintiff's Exhibit 5.*

| Name | Date sold |
|---|---|
| Medical Officer in Charge<br>U. S. P. H. S. Treasury Dept.<br>Hot Springs, Arkansas | 5/26/39 |
| Columbia University<br>New York City | 6/1/39 |
| Washington University<br>St. Louis, Mo. | 6/2/39 |
| University of Iowa Hospital<br>Iowa City, Iowa | 6/15/39 |
| The Johns Hopkins University<br>Baltimore, Maryland | 6/20/39 |
| U. S. National Institute of Health<br>Washington, D. C. | 7/5/39 |
| Moreno Borlido & Cia.<br>Rio de Janeiro, Brazil | 7/6/39 |
| City of St. Louis Psychopathic Hospital<br>St. Louis, Mo. | 7/10/39 |
| Typhus Research Laboratory<br>U. S. Public Health Service<br>Albany, Georgia. | 7/11/39 |
| [183] Grasslands Hospital<br>Valhalla, New York | 8/ 3/39 |
| Sydenham Hospital<br>Baltimore, Maryland | 8/21/39 |
| State of Michigan<br>Dept. of Health<br>Lansing, Michigan | 10/17/39 |
| Health Department<br>City of St. Louis, Mo. | 12/11/39 |

*Plaintiff's Exhibit 5.*

| Name | Date |
|---|---|
| Presbyterian Hospital<br>Philadelphia, Pa. | 12/20/39 |
| Fromm Laboratories<br>Grafton, Wisconsin<br>Dr. Green | 12/28/39 |
| Roosevelt Hospital<br>New York City | 1/ 2/40 |
| Fromm Laboratories<br>Grafton, Wisconsin | 1/29/40 |
| State Hygienic Laboratory<br>University of Nevada<br>Reno, Nevada | 1/31/40 |
| Rockefeller Institute<br>New York, N. Y. | 2/ 8/40 |
| E. R. Squibb & Sons<br>New Brunswick, N. J. | 2/ 8/40 |
| Veterans Admin.<br>Aspinwall<br>Pittsburgh, Penna. | 2/24/40 |
| Mt. Sinai Hospital<br>New York, N. Y. | 3/ 4/40 |
| Swiss Serum & Vaccine Institute<br>Berne, Switzerland | 3/21/40 |
| [184] Dept. of Agriculture<br>Food and Drug Admin.<br>Div. of Bacteriology<br>Washington, D. C. | 4/ 4/40 |
| Texas State Board of Health<br>Austin, Texas | 4/16/40 |

*Plaintiff's Exhibit 5.*

| Name | Date |
|---|---|
| University of Virginia<br>Dept. of Internal Med.<br>University, Virginia | 4/17/40 |
| The Leonard Wood Memorial<br>Culion, Philippine Is. | 5/20/40 |
| Animal Disease Station<br>Beltsville Research Center, Bureau of Animal Industry<br>Dept. of Agriculture<br>Beltsville, Maryland | 5/22/40 |
| Medical Center for Federal Prisoners<br>Springfield, Missouri | 5/28/40 |
| Public Health Service<br>Rocky Mt. Laboratory<br>Hamilton, Montana | 6/ 1/40 |
| Director, Natl. Institute of Health<br>U. S. Public Health Service<br>Bethesda, Maryland | 7/ 1/40 |
| Mount Zion Hospital<br>San Francisco, Calif. | 8/12/40 |
| Colorado Medical School<br>University of Colorado<br>Denver, Colorado | 8/13/40 |
| [185] Johns Hopkins University School of Medicine<br>Dept. of Preventive Medicine<br>Baltimore, Maryland | 10/ 4/40 |

*Plaintiff's Exhibit 5.*

| Name | Date |
|---|---|
| Harlem Hospital<br>New York, N. Y. | 10/ 9/40 |
| Serum Research Labs. Inc.<br>Detroit, Michigan | 11/ 5/40 |
| The University of Nebraska<br>College of Medicine<br>Omaha, Nebraska | 11/19/40 |
| Central Scientific Co.<br>Cambridge A. Station<br>Boston, Mass.<br>for<br>American Red Cross<br>Harvard Univ. Hospital<br>Unit for London | 12/ 4/40 |
| University of Missouri<br>Columbia, Missouri | 12/ 5/40 |
| Veterinary Station Hospital<br>Front Royal Q. M.<br>Depot (Remount)<br>Front Royal, Virginia | 12/23/40 |
| Lederle Labs.<br>Pearl River, N. Y. | 1/ 2/41 |
| Duran, Garcia & Co.<br>Lisbon, Portugal | 2/19/41 |
| State of Idaho<br>Dept. of Pub. Health<br>Boise, Idaho | 5/22/41 |
| [186] The Upjohn Company<br>Kalamazoo, Michigan | 6/ 5/41 |
| Imperial Veterinary<br>Research Institute<br>Muktwswar, Calcutta,<br>India | 6/ 9/41 |

*Plaintiff's Exhibit 5.*

| NAME | DATE |
|---|---|
| University of Michigan<br>Ann Arbor, Michigan | 6/ 9/41 |
| U. S. Dept. of Agriculture<br>Eastern Regional Research Lab.<br>Ardmore & Mermaid Ave.<br>Wyndmoor, Pa. | 6/25/41 |
| King Institute<br>Guinay, Madras, India | 6/26/41 |
| Public Health Service<br>14th Ave. & Lake St.<br>San Francisco, Calif. | 7/ 2/41 |
| Venereal Disease<br>Research Laboratory<br>U. S. Marine Hospital<br>Staten Island, N. Y. | 7/ 3/41 |
| State Health Dept.<br>State Office Bldg.<br>Richmond, Virginia | 8/ 1/41 |
| Tennessee Valley Authority<br>Sheffield, Alabama | 10/24/41 |
| University of Wisconsin<br>Madison, Wisconsin | 11/17/41 |
| Massachusetts Memorial Hospital<br>Boston, Mass. | 12/17/41 |
| [187] Ministerio de<br>Sanidad y Assistencia Social<br>La Guaria, Venezuela | 2/12/42 |

*Plaintiff's Exhibit 5.*

| Name | Date |
|---|---|
| Adromachus Corp.<br>Long Island City, N. Y.<br>(for their Mexico office) | 3/11/42 |
| Ohio State University<br>Columbus, Ohio | 3/16/42 |
| Veterinary Department<br>Nigeria Supply Board<br>Government of Nigeria<br>West Africa | 3/19/42 |
| E. R. Squibb & Sons<br>New Brunswick, N. J. | 4/ 3/42 |
| Bergen County Hospital<br>Ridgewood, N. J. | 4/21/42 |
| U. S. Naval Medical Center<br>Bethesda, Maryland | 5/18/42 |
| The Children's Hospital<br>Cincinnati, Ohio | 8/11/42 |
| Rockefeller Foundation<br>New York, N. Y.<br>(for their Institute in Brazil) | 9/28/42 |
| Ambassade de Belgique<br>New York, N. Y.<br>(for Belgian Congo) | 10/ 5/42 |
| New York Medical Dept.<br>U. S. Army<br>Marietta, Penna. (for export) | 12/ 4/42 |
| [188] Medical Supply Officer<br>Fort Bragg, N. C. | 12/10/42 |

*Plaintiff's Exhibit 5.*

| Name | Date |
|---|---|
| Henry Phipps Institute<br>Philadelphia, Pa. | 12/30/42 |
| Instituto Pasteur<br>de Lisboa<br>Lisbon, Portugal | 3/ 8/43 |
| Jewish Hospital of<br>Brooklyn<br>Brooklyn, New York | 3/ 3/43 |
| The Upjohn Co.<br>Kalamazoo, Michigan | 3/25/43 |
| Rockefeller Foundation<br>49 W. 49th St.<br>New York, N. Y.<br>(for export to Algiers) | 5/25/43 |
| Lakeside Laboratories<br>Milwaukee 1, Wisconsin | 7/26/43 |
| Columbia University<br>New York City, N. Y. | 8/ 9/43 |
| Washington University<br>Dept. of Pathology<br>St. Louis, Mo. | 8/17/43 |
| Syracuse University<br>College of Medicine<br>Dept. Bact. & Parasitology<br>Syracuse, New York | 4/24/43 |
| The Upjohn Company<br>Kalamazoo, Michigan | 9/ 1/43 |
| J. T. Baker Chemical Co.<br>Phillipsburg, N. J. | 9/ 3/43 |
| [189] Cheplin Biological<br>Laboratories<br>Syracuse, New York | 10/22/23 |
| ditto | 10/13/43 |

## *Plaintiff's Exhibit 5.*

| NAME | DATE |
|---|---|
| Bronx Hospital<br>Bronx, New York | 10/14/43 |
| Heyden Chemical Co.<br>Garfield, N. J. | 10/15/43 |
| Lambert Pharmacal Co.<br>St. Louis, Missouri | 10/20/43 |
| Bayer Co. Div. Sterling Drug Co.<br>Rensselaer, N. Y. | 10/20/43 |
| Ayerst, McKenna & Harrison<br>Montreal, Canada | 10/28/43 |
| The Upjohn Company<br>Kalamazoo, Michigan | 11/ 4/43 |
| Tulane Univ. of La.<br>New Orleans 15<br>Louisiana | 11/19/43 |
| Ciba Pharmaceutical Products Inc.<br>Summit, N. J. | 11/22/43 |
| Purdue University<br>Lafayette, Indiana | 12/14/43 |
| Mayer & Myles Labs.<br>Philadelphia, Pa. | 12/23/43 |

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# A NEW AND ECONOMICAL DESICCATING PROCESS PARTICULARLY SUITABLE FOR THE PREPARATION OF CONCENTRATED PLASMA OR SERUM FOR INTRAVENOUS USE: THE ADTEVAC PROCESS *

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A PRACTICAL and inexpensive desiccation process is vital to the development of the widespread use of plasma and serum (ordinary or convalescent) because it solves two main problems in this field. First, storage difficulties practically cease to exist. While it is true that liquid serum may be stored, even without refrigeration as emphasized recently by Levinson,[1] there is no margin of safety. Contamination with but one bacterium could quickly spoil this excellent culture medium if stored without refrigeration. Also at room temperatures, the antibodies, prothrombin and other valuable biological properties rapidly deteriorate. By contrast, serum stored in the dry so-called lyophilic form will not support bacterial growth at any temperature, and in addition all the biological properties are preserved to a remarkable degree.

Under optimum conditions, no limit for the preservation of antibodies has yet been determined but it appears almost certain that immune sera can be preserved for many years. Even the highly labile complement has been successfully preserved by Kolmer[2] for 13 months. The possibility of almost indefinite preservation of potent convalescent sera, accomplished by an inexpensive method, brings the serum center and all its advantages within the scope of the average large hospital. With the necessity for rapid "turnover" of serum eliminated, such serum centers need not be limited to great centers of population as in the past. The safe, yet indefinitely long storage of whole plasma, possible in the dehydrated state, should also make practical

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From the Department of Pathology, Hospital Laboratory Division, Baylor University College of Medicine.

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the extensive use of the plasma treatment of shock in wartime. Being dry, the plasma could be contained in small metal ampoules or cans to facilitate shipment and handling.

The second problem solved by desiccation is the production of an ideal hypertonic solution for intravenous use, namely, concentrated plasma or serum. When the dried plasma is dissolved in distilled water any desired concentration from four times normal down to normal or even more dilute than normal (hypotonic) can be made as desired. This not only permits unusual flexibility, but also opens up new therapeutic possibilities in the regulation of fluid balance, blood volume and pressure, as yet only partially explored. Furthermore enough protein can be given as concentrated plasma with a large syringe (100 c.c.) at 12 to 48 hour intervals to sustain blood protein levels when food cannot be taken by mouth or when for other reasons protein levels are not maintained as in cirrhosis, cachectic states, etc.

These therapeutic advantages, peculiar to the intravenous use of human plasma or serum of various concentrations, have not as yet been generally appreciated. This has been due in a large measure to the limited supply of such plasma. Two reasons for this limitation have been apparent: first, the difficulty of obtaining a sufficient amount of blood, and second, the expense or complexity of available desiccating processes by which storage and concentration can be accomplished. Accordingly, it is the purpose of this paper to describe a new desiccating process employed at Baylor University Hospital for this particular use, and to report its routine operation in connection with a Blood Bank, to provide an adequate supply of plasma of any desired concentration up to four times normal.

In reviewing the development of desiccation of biological substances, it is of interest to note that definitely improved methods of desiccation have been followed by new and multiplied uses. On this basis two periods can be made out. In the first, desiccation was used on a very small scale for a limited amount of research and special teaching. In the second, research and teaching uses were expanded and desiccation was applied to preservation of convalescent sera in a few large centers. A third period, marked by the general preservation and use of whole plasma of various concentrations attained by means of desiccation, seems to be foreshadowed by the growing appreciation of the value of supporting blood protein levels, and of the use of concentrated plasma for its hypertonic effects.

Shakell[3] in 1909 discovered the basic principle of vacuum desiccation from the frozen state, which is so essential to the production of a highly soluble product with retention of its original properties. He also encountered the basic problem of this type of dehydration, namely, how to deal with the huge volume of water vapor released from only a few cubic centimeters of ice under the high vacuum conditions necessary to this process, a volume far too great for any pump to handle. Shakell solved this problem by chemical absorption and set a precedent followed by many investigators since. His choice of sulphuric acid for a desiccant as well as the design of his machine definitely limited its use. Other workers using chemical absorption were Elser, Thomas and Steffen,[4] and Greaves and Adair,[5] who improved machine design but found their methods severely restricted by the characteristics of the chemicals used. In general these difficulties were insufficient speed of absorption, dilution, scum formation, or other changes retarding the pickup of water vapor as the process proceeded. These difficulties increased with any increase in size of the apparatus. This, and the expense involved in using new desiccant each time, limited the use of these processes. In quality, however, satisfactory products were obtained. Elser, for example, was able to preserve cultures of meningococci and gonococci in viable condition for 18 years.

Greater possibilities in the field of desiccation were opened up when Elser introduced the use of very cold surfaces for the condensation of water vapor as ice. He first used $CO_2$ snow (—70° C.) then mechanical refrigeration with resulting temperatures of —34° C. These low temperatures were held in a refrigeration line within the manifold and resulted in a flow of water vapor from the frozen material being processed, to the refrigerator tubing where it was bound as super cold ice. This vapor flow was a result of the lower vapor pressure of the ice on the tubing in comparison to the vapor pressure of the ice in the ampoule. Elser also appears to have been first not only to employ manifolds with compression joint connections, but also to process large quantities of biologicals in original containers and to vacuum seal directly from the machine. Flosdorf and Mudd[6] employed similar principles in their machine, using dry ice in methyl cellusolve for the condenser refrigerant and effected an improvement by removing the cold surface from the manifold to a connected condenser chamber.

These improvements were followed by reports of clinical use of convalescent serum preserved and concentrated by the technic of Flosdorf and Mudd. McGuiness, Stokes, and Mudd[7] described good results in the use of convalescent sera preserved by desiccation. In a large series of cases results were found to be comparable with regular liquid serum, both in prophylaxis and treatment, but five fairly severe febrile reactions were observed. Stokes, Mudd, Arddy, Eagle, Flosdorf, and Lucchesi[8] made a similar report of the effective use of convalescent serum preserved by desiccation. Mudd, Flosdorf, Eagle, Stokes, and McGuiness[9] reported not only additional information on the keeping qualities and use of desiccated convalescent serum, but also detailed their technic of collections and filtration for intravenous use.

The value of the hypertonic qualities of concentrated serum was recognized in the experimental work of Bond and Wright[10] in the treatment of shock by its intravenous administration. Hughes, Mudd, and Strecker[11] reported the advantage of intravenous concentrated serum for the reduction of increased intracranial pressure. The effect of concentrated serum on the spinal fluid pressure was studied further in animals by Wright, Bond, and Hughes.[12] Aldrich, Stokes, Killingsworth, and McGuiness[13] used concentrated serum with good results to initiate diuresis in the treatment of nephrosis. Ravdin[14] employed lyophil serum both concentrated and normal for the prevention and correction of hypoproteinemia after gastric operation. However, he hesitated to recommend it because of the frequency with which he encountered reactions.

The exact method of preparation of the serum used in the above reports was not always stated; presumably a low temperature condenser method was employed. At best, this method is too expensive for general use. A very much cheaper process, the Cryochem, was described by Flosdorf and Mudd[15] in 1938. They resorted to chemical absorption but with much greater success than earlier workers because of their choice of a much more suitable chemical, specially prepared anhydrous calcium sulphate, known as "Drierite." This process, although the most economical to date, still has serious drawbacks to be discussed later. A recent work on desiccation is that of Greaves and Adair.[16] They employ the older method of cold surface condensation of the water vapor but make important contributions to the study of the thermodynamics of desiccation. Bauer and Pickels[17] described a modification of the Flosdorf and Mudd Lyophil Machine in which they desiccated yellow fever virus. Continued frozen condition of the virus was assured by keeping it during processing in a hardening cabinet at — 18° C.

## THE ADTEVAC PROCESS: DESCRIPTION OF PRINCIPLE AND OPERATION

In attempting to build a machine at Baylor to operate with the Blood Bank, and suitable for the desiccation of large quantities of normal plasma as well as convalescent serum, we early realized the need, not for a different type of machine, but for an entirely new process. As a result, the older methods of cold surface condensation and chemical action were abandoned and experiments with a physical process, adsorption, were undertaken. The entirely new problems encountered in its use for this purpose were satisfactorily handled by the application of the principles of thermodynamics and proper engineering practice and design.

Like other processes, the one herein reported accomplishes desiccation from the frozen state by means of vacuum and removal of water vapor. The removal of water vapor, however, is accomplished in a manner entirely new in this field, namely by controlled adsorption. The operation can best be explained by reference to a simplified diagram of the machine, figure 1. The serum is contained in an ampoule (1) attached by a compression joint (2) and flexible metal bellows (3) to a vacuum tank (7) in which the adsorbent (8) is placed. When the vacuum pump (9) has sufficiently reduced the pressure in the system, rapid boiling occurs in the serum as dissolved gases, such as $O_2$, $N_2$, $CO_2$, etc., are released. During this phase the flexible metal bellows (3) permits lowering of the ampoule. The violence of the degassing process is controlled by admitting small amounts of air through a valve (4). This air is sterilized by passage through a suitable filter (5) and sterility maintained in the ampoule and manifold by properly locating a valve so that the direction of flow is always away from sterile area to non-sterile. When degassing is sufficiently complete the valve (4) is kept closed and when the vacuum, as measured by a special gauge[12] goes to about 1500 (absolute pressure in microns) snap freezing occurs spontaneously as a result of the rapid evaporation. Prior to freezing the ampoule is raised to a horizontal position to present a maximum evaporating surface. Evaporation (more correctly sublimation) continues from the frozen surface until the serum has reached the desired dryness.

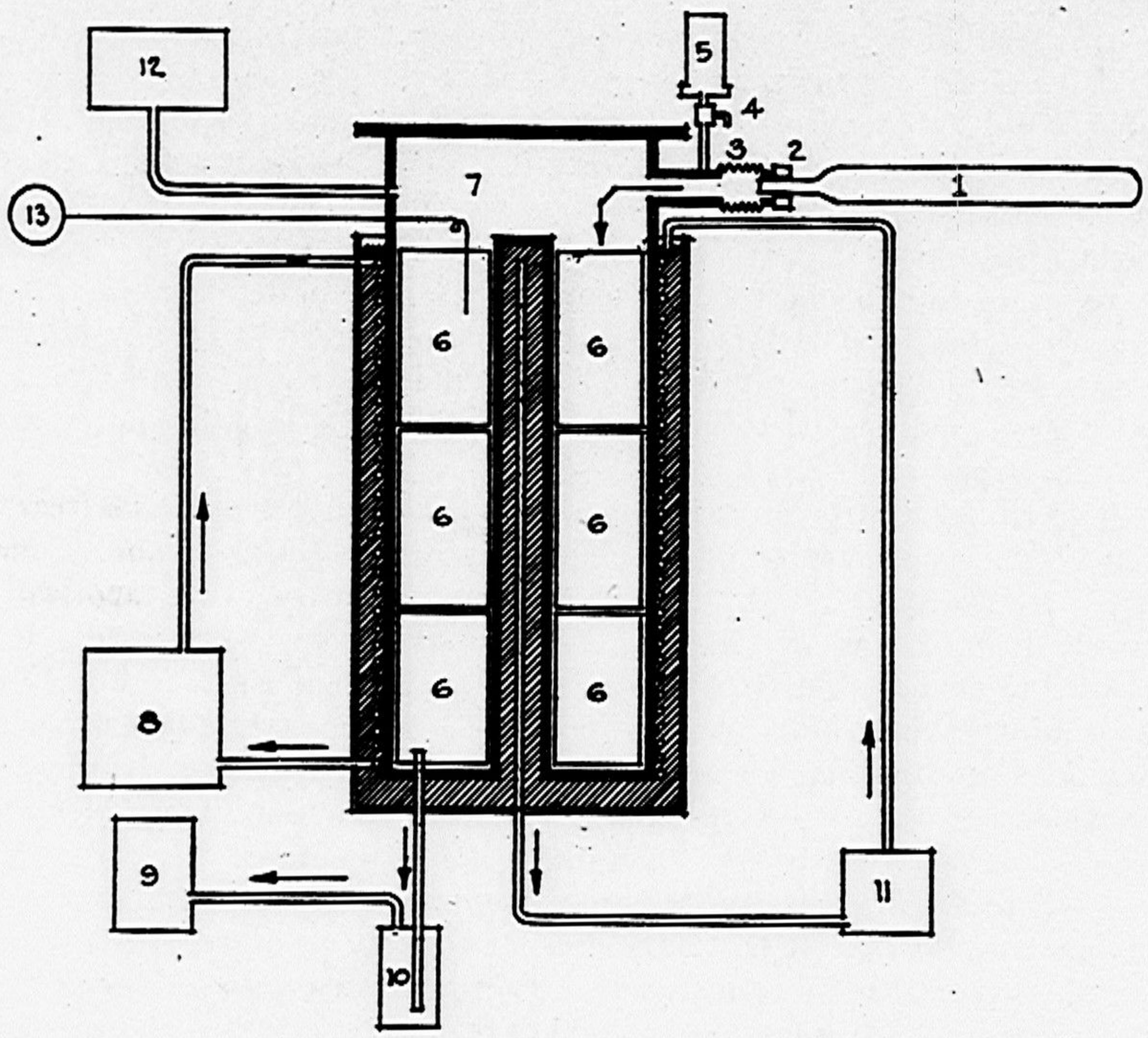

FIG. 1. Diagrammatic sketch of equipment.

In order to appreciate the advantages of controlled adsorption in our process it is necessary to understand the problem of water vapor removal. This apparently simple problem has presented most of the difficulties in all prior methods of desiccation.

In order to freeze and maintain the substance being processed in the frozen state, a sufficiently low vacuum must be held in the system. A vacuum pump is used to remove the non-condensible gases (air), from the system. Evaporation of water is accelerated as the pressure is reduced and in a relatively short time as the pump continues to remove the air, the system will be almost entirely filled with water vapor with continually diminishing minimal quantities of air. When the point is reached where the air is eliminated and the system entirely filled with water vapor * a definite relation of temperature to pressure is found. This is according to the well known laws governing the pressure temperature relation of saturated steam or water vapor.

It is well known, for example, that the temperature in a steam sterilizer is related directly to the steam pressure and to the pressure only. Less familiar is the fact that the same relation in respect to water vapor holds as the pressure is reduced well below atmospheric levels. Below atmospheric levels the vacuum is conveniently expressed as absolute pressure in terms of the height of a column of mercury, as read by a special instrument. For example, at 9.1 mm. of mercury the temperature of saturated vapor is 10° C. This also means that at this pressure water boils at 10° C. At pressures below 4.5 mm. the temperature will be below the freezing point of water and under these conditions water in the system will have to be either in the form of ice or vapor.

This temperature pressure relationship explains why spontaneous freezing occurs in the ampoule when the absolute pressure falls below 4.5 mm. of mercury. It also indicates why the plasma remains frozen, with vaporization directly from the ice crystals continuing until dryness is attained. It is essential to note that once the water becomes vapor it must be regarded as a gas, subject to the laws of gases. As a consequence, we find that under the conditions of operation, for instance at an absolute pressure of 181 microns, the volume of water vapor resulting from the evaporation of one liter (one kilo) of water would be 4,670,930 liters. This indicates that no vacuum pump of reasonable size could remove the water vapor at sufficient speed. Failing to do this, the high vacuum necessary for the process obviously could never be attained, and freezing could not occur. Consequently, the necessity became apparent for some provision other than the vacuum pump, to remove the water vapor in the process.

In searching for a suitable substance to bind water vapor an adsorbent such as silica gel was suggested by two outstanding properties demonstrated by its use in the field of air conditioning. The first of these is its ability to take up very large quantities of water vapor up to 30 per cent in weight or more, and the second is the relative permanence of such substances. Since the union to water is physical, no appreciable deterioration occurs, upon drying off the water by heat, cooling and re-using. With reasonable care the adsorbent can be used indefinitely.

At this point a brief consideration of adsorption seems desirable. Adsorption is defined as the concentration of substances out of liquid or gaseous mixtures on the surfaces of liquids or solids. Many solid materials such as silica gel, charcoal, activated alumina, ferric hydroxide gel and others possess the power to adsorb gases and vapors to an unusual degree. According to

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* Due to inevitable small leaks, a perfect saturated vapor condition is never actually attained. For practical purposes, however, it may be so considered.

Lednum[18] the mechanism by which this surface adsorption takes place is not actually known as the effects are beyond the powers of the most powerful microscope but a careful study by numerous investigators indicates the probable combination of three separate effects.

The first of these is probably the formation of a layer of the adsorbed

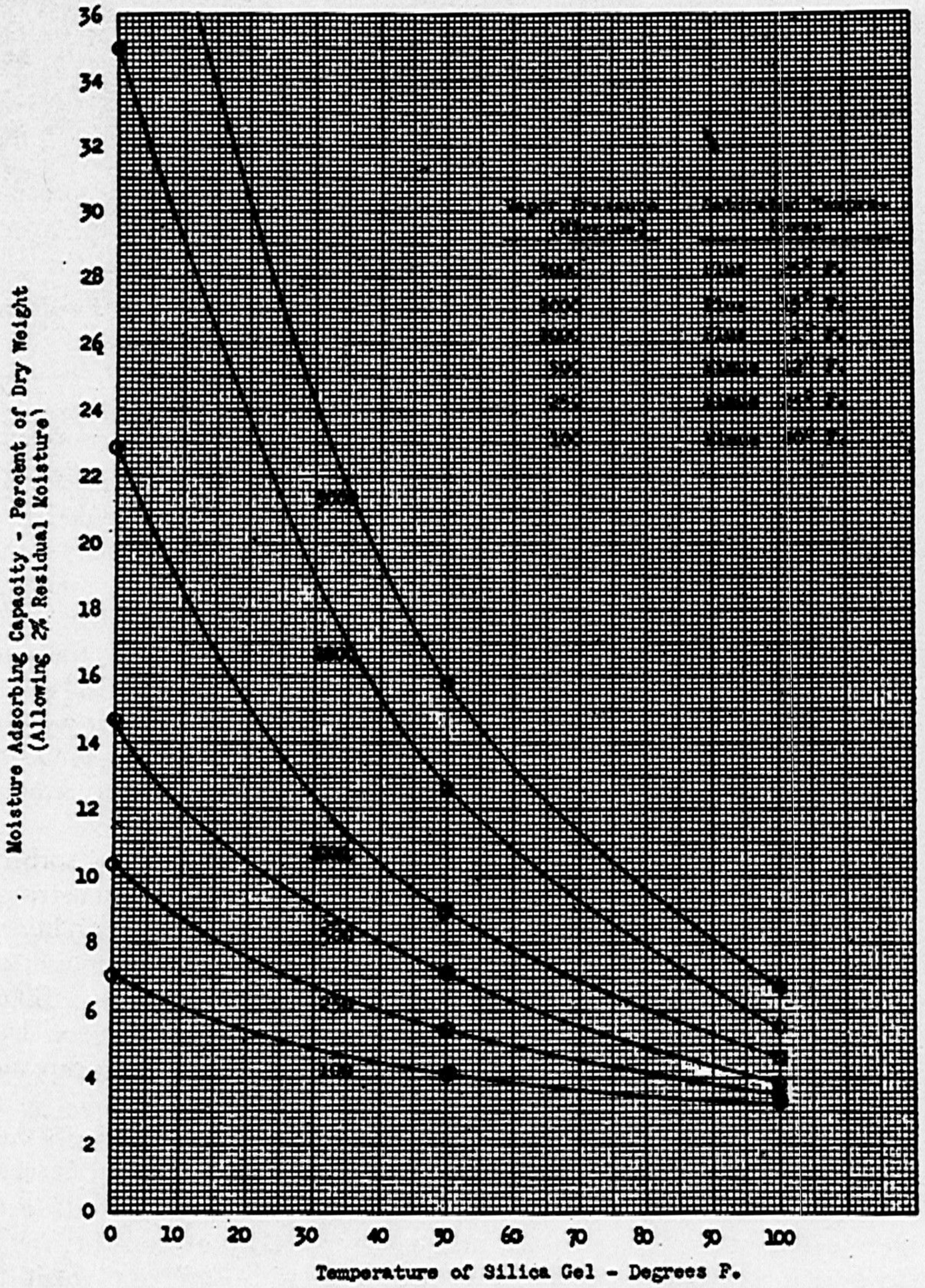

FIG. 2. Capacity-temperature relation of silica gel at vapor pressure (microns) as indicated on curves. Curve No. 2.

vapor one molecule deep on the surface of the solid. The second effect consists of the formation of a second layer of vapor of variable thickness held by the molecular attraction between the solid and the vapor, the thickness depending upon the strength of the attraction. The third effect is that of collection on the surface of submicroscopic capillary openings, of the more or less condensed layer of vapor. The three effects are cumulative, and adsorbing agents possess them in varying proportions to a greater or less degree. The amount of vapor adsorbed is, therefore, a function of the vapor pressure. It is evident that it must be also a function of the temperature. Change in either vapor pressure or temperature alters what may be called the " ease of condensation " of the vapor.

Reference to figure 2 shows that the capacity of a typical adsorbent (silica gel) to adsorb water vapor is dependent not only upon the vapor pressure to which the gel is exposed, but also upon the temperature of the gel itself. At low temperatures the capacity is greatly increased, while rising temperatures in the adsorbent are accompanied by diminished capacity to take up water vapor. This fact is of great practical importance since a definite amount of heat is liberated when the adsorbent takes up water called the heat of adsorption, which by raising the temperature of the adsorbent tends to reduce its capacity for further binding of water. In our machine this heat is removed by a small standard refrigeration unit. The actual transfer of heat can easily be accomplished in several ways according to the engineering design, by providing suitable metal pathways.

By this arrangement of refrigeration and heat transfer it is not only possible to remove the heat generated during the operation of the process, but it is also possible to cool the adsorbent and improve its effective capacity. Furthermore, since the greatest flow of vapor occurs early in the process, just prior to freezing, with consequent generation of unusual amounts of heat, it is best to pre-cool the adsorbent to accommodate this temporary heavy load.

If desired the plasma may be pre-frozen in the ampoules. This of course is desirable when desiccating small amounts such as are employed in storing complement, cultures, and in other laboratory uses.

As Greaves and Adair [16] have pointed out, the desiccation time can be greatly shortened by applying heat to the substance being processed, especially in the earlier stages. This heat is translated into an increased flow of vapor from the frozen surface, but if this increased flow can be removed rapidly by the desiccant the pressure in the ampoule need not rise significantly and as a consequence the temperature will be substantially unchanged. In the Adtevac process advantage can be taken of this fact due to its ability to take up large volumes of water vapor rapidly. This represents a definite advance in speed of processing since earlier methods were easily overloaded when acceleration of evaporation by heat was attempted, as Greaves and Adair [16] showed.

For the desiccation of large quantities of ordinary plasma we have used ampoules of great capacity, usually 500 c.c. We have not observed the restrictions laid down by Flosdorf and Mudd [6] in respect to ratio of fluid volume to surface. On freezing, stratification occurs with great increase of surface with satisfactory rapid drying. The size of the ampoule neck has been kept as large as possible to keep restriction of vapor flow to a minimum. The use of metal bellows for flexible coupling has also eliminated the constriction which rubber tubing always imposes. With 200 c.c. quantities per ampoule satisfactory drying of plasma has been accomplished in six hours.

Figure 3 is a photograph of the machine now in use.

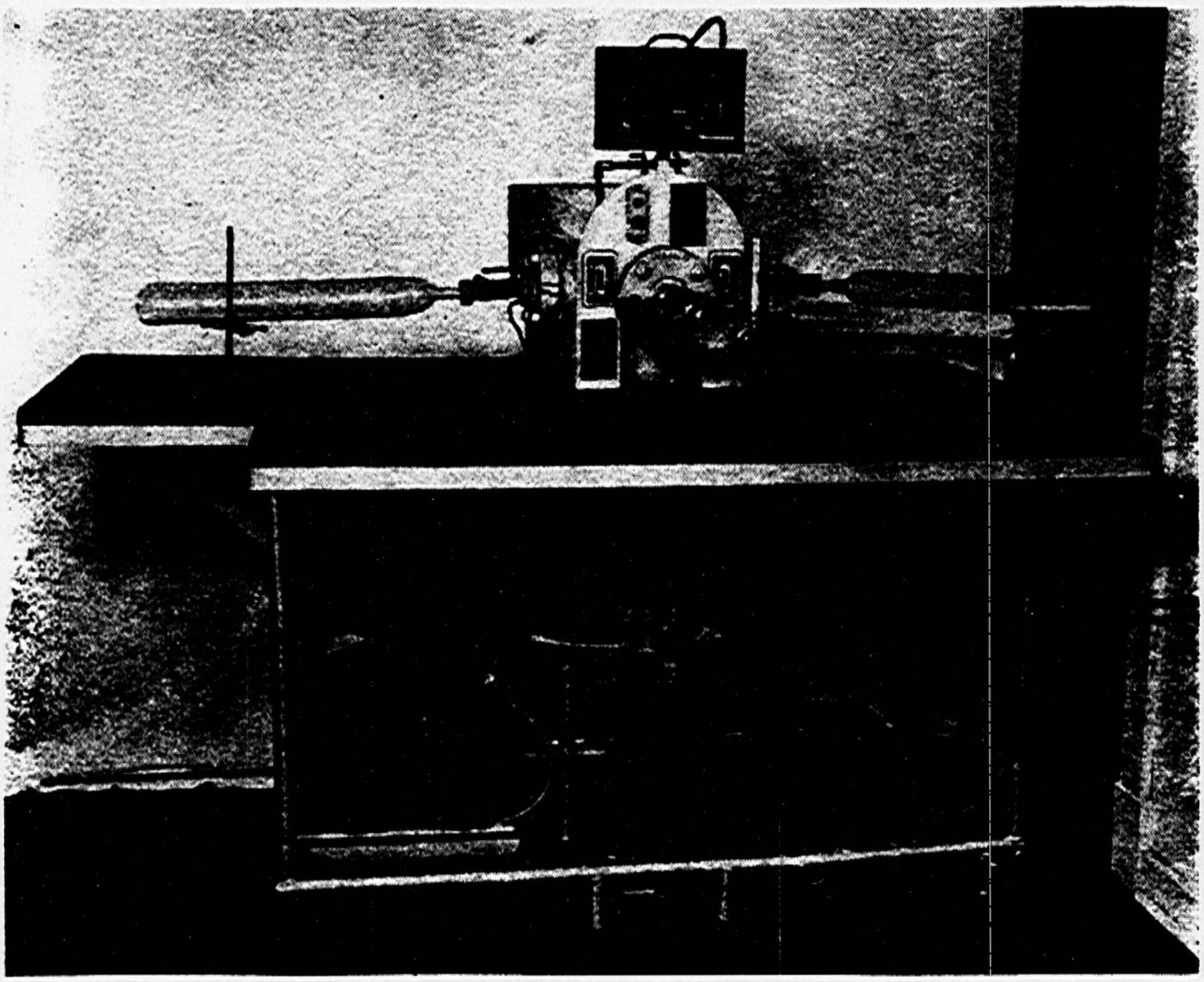

FIG. 3.

## DISCUSSION

In comparing our process with prior methods we have considered the following points: quality of product, economy of operation, practical maximum capacity, reliability, ease of operation, and initial cost.

The quality of materials can be estimated most satisfactorily by extensive use. Our clinical results from the intravenous administration of plasma desiccated by this process are briefly summarized in a later section of this paper. Another test of quality, however, is the determination of residual dryness according to the method of Flosdorf and Webster. They state that a residual moisture of 1 per cent or less is satisfactory for biological use. We have found that the possible dryness obtained varied with the moisture load imposed on the adsorbent. However, even with maximum loads it was found possible to attain moisture analyses of less than 1 per cent. However, when plasma was further subjected to desiccation with a new dry charge of adsorbent substituted for the already used, thus operating the process in two stages,* the residual moisture is almost too small for accurate analysis. For example, under these conditions moistures of .03 per cent and less are obtained.

The chief advantage of the new process is the economy of operation. Unlike earlier methods using chemical absorption, the Adtevac process does not require the frequent replacement of expensive desiccants. Furthermore, much larger quantities of water for a given weight of adsorbent can be taken up at one operation. This emphasizes the disadvantages of chemical union with water vapor as contrasted with adsorption, since by our method as high as 14 per cent capacity has already been realized. Theoretical considerations indicate a considerably higher capacity can be attained under optimum conditions.

The limited space usually allotted to hospital laboratories makes it desirable to have a maximum capacity for unit size. In this respect the new adsorption process enjoys a marked advantage due to the high capacity per unit weight.

The actual process has proved itself reliable and easy to operate over a period of many months. It should be noted that the refrigeration unit employed in this machine is a small commercial unit operating under ordinary temperatures and is not to be confused with the huge refrigeration system employed in the direct cold surface condensation methods where operation must be conducted in the difficult sub-zero region.

Experiments now in progress indicate another advantage of controlled adsorption for desiccation. The great capacity to take up water vapor rapidly, permits the application of relatively large quantities of heat to the ampoules without melting the contained material. This drives off the vapor more rapidly and greatly shortens the desiccation time. Also important is the margin of safety that this capacity gives under normal operating conditions when substances such as virus preparations are to be desiccated, since evaporation will continue with such speed that the temperature will remain well below freezing until the desiccation is essentially complete.

This is a point of greatest importance, if overloading and thawing or even near thawing is to be avoided. Proper attention to these considerations would probably eliminate the need for cumbersome and expensive external refrigeration of the containers or ampoules.

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* Note: For large capacity machines this two-stage operation can be used routinely, by employing two desiccant chambers. These are both connected to the manifold by electrically operated remote control valves contained within the large tube uniting the two chambers. Such a valve presents no difficulty because of the slight difference in absolute pressure in the two chambers.

Estimates of the initial cost of the machine are not as yet complete, but it is apparent that on a capacity basis it should be considerably less than machines now available.

## Source of Plasma Supply

The Blood Bank constitutes an ideal organization to obtain an adequate supply of plasma. Donors are required to give an amount of blood sufficient to produce the amount of plasma given. For example since blood is about one-half plasma and since a four times concentration is effected, it would theoretically require eight times the volume of blood to produce the maximum concentration of serum. Since some plasma is lost in separation from cells, and in filtration, a 10 to 1 ratio is more nearly correct.

Blood given for the purpose of replacing plasma is ordinarily kept in the Blood Bank up to the maximum of 10 days. This is of value to the Blood Bank in keeping an adequate supply of all types of whole blood available. Conversely, the Blood Bank is of assistance to the plasma department since all blood from the bank when outdated at the tenth day is processed and stored.

In preparing the plasma a technic similar to that of Mudd, Flosdorf, Eagle, Stokes and McGuiness [9] was used at first. More recently a new technic, eliminating the restrictions imposed by the type specific agglutinins has been adopted as routine. The removal of agglutinins is accomplished by adsorption on red cells as a result of pooling the different types of whole blood and immediately separating the plasma.

Accompanying hemolysis is kept at a minimum by working rapidly at low temperature. Hemolysis is also retarded by the low complement content of 10 day old blood. However, some hemolysis has occurred with definite coloring of the plasma. An approximate determination by the Newcomer method using a one to four dilution has shown a maximum of 0.3 grams per 100 c.c. of plasma. The agglutinin titer has varied from 0 to 1 : 1.

Since we have observed no evidence whatever that hemolysis causes reactions in six months' experience with blood banking we did not hesitate to use plasma containing small amounts of hemoglobin. This is contrary to the commonly accepted opinion in blood banking. However, no febrile or other type of reaction (with the exception of two transient urticarial manifestations) has been observed in the use of such plasma in concentrated form.

Following a suggestion of Levinson,[19] we have omitted filtration of plasma after desiccation and reconstitution as a liquid. Clinical results have been unchanged, and loss of valuable concentrated plasma in the filter is thus eliminated. Seitz filtration of plasma separated from pooled blood is performed prior to processing.

Plasma intended for long storage is vacuum sealed in the original ampoules. For current routine use, the plasma is redissolved in pyrogen free water to make a four times concentration, placed in small Erlenmeyer flasks, and kept frozen at — 18° C. For use this plasma is melted in a 37.5° C. water bath and given with a large syringe (100 c.c.).

## INDICATIONS AND CLINICAL RESULTS

The plasma has been given in concentrated form (1) to replenish deficiencies in blood protein, (2) to build up or sustain blood volume, (3) for hypertonic effect in reducing edema, and (4) in a miscellaneous group. When hypertonic effects were not desired or if additional fluid was needed, a suitable amount of saline was given after the administration of the concentrated plasma.

To date 66 doses of concentrated plasma have been given intravenously to 45 individuals. In this series no definite febrile reactions were encountered in any instance. In fact, examination of charts of these patients shows that febrile patients have as a rule exhibited a substantial drop in temperature of 1 to 1½° F. depending somewhat upon the height of the fever. As a rule the plasma was given over a period of 10 to 15 minutes, but on some occasions, particularly in emergency, such as in severe shock, the entire dose has been given as quickly as 1½ minutes. The largest dose given at one time was 170 c.c. This would be equivalent in protein content to 1360 c.c. of whole blood. This is in accord with the practice of not exceeding 200 c.c. for a single dose to adults.

A detailed analysis of clinical results will be reported later when a larger, more complete series of cases has been compiled. However, even in this small series fairly obvious and definite advantages in the use of concentrated whole plasma stand out. The best results were obtained where there was local edema combined with reduced blood volume in shock. This was to be expected since concentrated plasma builds up blood volume by withdrawing water from the tissues. Such cases as shock with head injuries have responded favorably.

In shock from acute hemorrhage (ruptured ectopic pregnancy) concentrated plasma has rapidly built up the blood pressure prior to transfusion and operation.

In a small series of burns, several very severe cases have responded to the use of concentrated plasma with the relief of shock and apparently accelerated healing with maintenance of blood protein levels. The plasma was given with or without additional fluid according to the patient's fluid balance. Concentrated plasma alone was employed to advantage when the patient was " water logged " from prior administration of large amounts of saline.

Blood protein levels have been successfully maintained by the intravenous administration of concentrated plasma in several cases where adequate diet could not be taken by mouth. These patients all showed improvement with increase in strength.

Convalescent sera have also been dried, stored and used with good results. Obviously time has not permitted the determination of the effect of prolonged storage.

The process has been entirely satisfactory for laboratory uses such as preservation and concentration of typing sera, preservation of cultures, positive sera, and thromboplastin extracts for the prothrombin test.

## Conclusions

1. A new and improved process for the desiccation of plasma, serum and biological substances from the frozen state is reported.
2. The thermodynamics of this type of desiccation are investigated and discussed.
3. The operation of this process in connection with a Blood Bank to assure an adequate supply of plasma for routine intravenous use is described.
4. A preliminary report of clinical results in a variety of conditions is given.
5. No febrile or other harmful reactions were noted in 66 successive intravenous administrations of concentrated plasma to 45 cases.

## BIBLIOGRAPHY

1. Levinson, S. O., Neuwelt, F., and Nechelese, H.: Human serum as a blood substitute in the treatment of hemorrhage and shock, Jr. Am. Med. Assoc., 1940, cxiv, 455–461.
2. Kolmer, J. A., Richter, C. E., and Lynch, E. R.: Lyophile complement fixation test for syphilis, Am. Jr. Clin. Path., 1938, viii, 522–528.
3. Shakell, F. F.: An improved method of desiccation with some applications to biological problems, Am. Jr. Physiol., 1909, xxiv, 325–340.
4. Elser, W. J., Thomas, B. A., and Steffen, G. I.: Desiccation of sera and other biological products (including microörganisms) in the frozen state with preservation of original qualities of products so treated, Jr. Immunol., 1935, xxviii, 433–473.
5. Greaves, R. I. N., and Adair, M. E.: Simple method for the preservation of sera by desiccation in frozen state without use of refrigerants, Jr. Hyg., 1936, xxxvi, 507–513.
6. Flosdorf, E. W., and Mudd, S.: Procedure and apparatus for preservation in "lyophile" form of serum and other biological substances, Jr. Immunol., 1935, xxix, 389–425.
7. McGuiness, A. C., Stokes, J., Jr., and Mudd, S.: Clinical uses of the human serums preserved by lyophil process, Jr. Clin. Invest., 1937, xvi, 185–196.
8. Stokes, J., Mudd, S., Arddy, R. L., Eagle, Harry, Flosdorf, E. W., and Lucchesi, P.: Use of lyophile human serums for prevention and treatment in infectious diseases, Am. Jr. Dis. Child., 1934, xlviii, 1428–1430.
9. Mudd, S., Flosdorf, E. W., Eagle, H., Stokes, J., and McGuiness, A. C.: Preservation and concentration of human serums for clinical use, Jr. Am. Med. Assoc., 1936, cvii, 956–959.
10. Bond, D. D., and Wright, D. G.: Treatment of hemorrhage and traumatic shock by the intravenous use of lyophile serum, Ann. Surg., 1938, cvii, 500–510.
11. Hughes, J., Mudd, S., and Strecker, E. A.: Reduction of increased intracranial pressure by concentrated solutions of human lyophile serum, Arch. Neurol. and Psychiat., 1938, xxxix, 1277–1287.
12. Wright, D. G., Bond, D. D., and Hughes, J.: Reduction of cerebrospinal fluid pressure by concentrated lyophile serum; further observations, Arch. Neurol. and Psychiat., 1938, xxxix, 1288–1293.

13. Aldrich, C. A., Stokes, J., Jr., Killingsworth, W. P., and McGuiness, A. C.: Concentrated human blood serum as diuretic in treatment of nephrosis; preliminary report Jr. Am. Med. Assoc., 1938, cxi, 129–133.

14. Ravdin, I. S.: Some recent advances in surgical therapeusis, Ann. Surg., 1939, cix, 321–333.

15. Flosdorf, E. W., and Mudd, S.: Improved procedure and apparatus for preservation of sera, microörganisms and other substances—cryochem-process, Jr. Immunol., 1938, xxxiv, 469–490.

16. Greaves, R. I. N., and Adair, M. E.: High-vacuum condensation drying of proteins from frozen state, Jr. Hyg., 1939, xxxix, 413–445.

17. Bauer, J. H., and Pickels, E. G.: Apparatus for freezing and drying virus in large quantities under uniform conditions, Jr. Exper. Med., 1940, lxxi, 83–88.

18. Lednum, J. M.: Theory of adsorption. Read before Industrial Gas Section, sixteenth annual convention and exhibition of Am. Gas Assoc., Atlantic City, N. J., (Oct. 30), 1934.

19. Levinson, S. O.: Personal communication.

From DEFENDANT'S EXHIBIT 1.

**Patented Nov. 8, 1932** **1,887,349**

# UNITED STATES PATENT OFFICE

WILLIAM A. HAMMOND, OF YELLOW SPRINGS, OHIO

## DRYING AGENT AND PROCESS OF MANUFACTURING SAME

**No Drawing.** **Application filed April 30, 1931. Serial No. 534,148.**

This invention relates to drying or desiccating agents and the process of manufacturing same and has for its primary object to provide a material having suitable physical and chemical properties to render it an efficient agent for the removal of water from other materials.

A further object of the invention is to provide a desiccating agent which may be manufactured from inexpensive materials and which may be regenerated to its original state after use.

Further objects and advantages will become apparent as the description proceeds.

In ordinary laboratory practice, (either routine or research), in the manufacture of certain materials, and in the control of humidity in chambers, vaults and buildings, it is frequently necessary to remove water from gases, liquids or solids, or to regulate the water or moisture content thereof. Such removal of water from other materials is usually accomplished by the use of one or more substances known variously as dehydrating agents, desiccating agents, or drying agents, and the specific physical and chemical properties of a given drying agent must usually be considered in connection with each particular drying operation.

The present drying agent consists of a form of sulphate of lime, or calcium sulphate, prepared from such original material and by such a process as will cause it to contain water, chemically combined in the form of water of hydration in amounts varying from 0 to 6.6 per cent by weight, and to possess the desirable physical and chemical properties which render it an efficient agent for the removal of water from other materials.

In preparing the drying agent, I prefer to use the snow-white natural mineral calcium sulphate dihydrate, known as gypsum, which contains approximately 20.9 per cent of water of hydration and which is practically free from foreign materials or impurities. However, good results may be obtained when a more crude gypsum is used, that is, a natural gypsum which has associated with it such natural materials as limestone, shale, clay, or other mineral substances. Also, good results may be obtained when the drying agent is prepared from the plaster of Paris casting moulds, new or old, used in the casting of ceramic bodies, from any set plaster of Paris mould, form, or casting, from plaster of Paris itself or commercial calcined gypsum, or from many of the so-called industrial by-products which contain appreciable percentages of hydrated calcium sulphate.

Having selected a suitable form of calcium sulphate as a raw material, I proceed to reduce the material to suitable physical form and size. When a natural mineral, or any form of cast set plaster of Paris, or calcined gypsum is used, the material is first cleaned and dried, after which it is crushed in a crusher designed to yield either a mixture of granules of all sizes from dust up to a predetermined maximum size, or a maximum proportion of some particular size with a minimum proportion of other sizes of granules. The crushed material is then screened through a series of screens; each screen in the series having successively smaller openings, such as 1, ½, ¼, ⅛, ⅛, 1/10, 1/20 inch. This process of screening yields portions of the original material in the form of fairly uniformly sized granules, and a mixture of coarse and fine powder.

When plaster of Paris or commercial calcined gypsum is used as the original material, the dry plaster of Paris or calcined gypsum may be mixed with water to form a paste, and this paste formed, cast, or extruded in the form of pieces, such as cylinders, cubes, etc., of any desired size and allowed to set and dry. My drying agent may be alternatively prepared directly from plaster of Paris or commercial calcined gypsum without wetting and casting, in which case the drying agent will be in the form of powder.

By the above described processes any form of calcium sulphate dihydrate (such as select or crude mineral gypsum, plaster of Paris casting moulds, casts or forms, plaster of Paris or commercial calcined gypsum itself) may be reduced or converted into uniformly sized granules or pieces or into powder as desired. With the physical form and size determined, it remains to develop in the material other desirable physical properties and such chemical properties as will cause it to become an efficient drying agent.

The further physical and chemical properties desired in an efficient drying agent are imparted to the above described material by a carefully controlled heat treatment. When calcium sulphate in any of the above-mentioned forms is heated, the water of hydration is expelled. The expulsion of the first three-fourths of the total water of hydration of the dihydrate (gypsum or plaster of Paris moulds) yields the ordinary plaster of Paris or commercial calcined gypsum, known as the half-hydrate. The expulsion of the last one-fourth of the total water of hydration of the dihydrate, or the expulsion of the total water of hydration of the half-hydrate, yields a product known as anhydrous calcium sulphate. This process of dehydration or expulsion of the water of hydration is not accompanied by any appreciable change in volume of the individual granules or particles; the dehydrated granule or particle is, therefore, permeated by fine pores. Furthermore, the process of dehydration while causing some lowering, by no means destroys, the resistance of the granules or particles to crushing and abrasion. The material may therefore be used and handled repeatedly without appreciable change in its granular form.

The chemical properties of anhydrous calcium sulphate, particularly with respect to its reactions with water, vary widely with the temperature and duration of heating used in its preparation. When heated at temperatures and for periods of time which are sufficient to effect practically complete dehydration, but below about 300 degrees centigrade, the product shows an extremely high chemical affinity for water. This product reacts instantaneously with water, liberating heat, and reverts to the form of the half-hydrate. The avidity with which this form of anhydrous calcium sulphate reacts with water to form the half-hydrate renders it a drying or desiccating agent of the highest efficiency. Thus, moist air or other gases allowed to stand or pass in contact with granules of the material are rendered practically free from water vapor. The water vapor remaining in air after drying in this way at 25° C. has been found to be only approximately 0.004 milligrams per liter. A moist solid stored in a sealed container such as a desiccator, or in any chamber or room, large or small, in which is placed a proper amount of this drying agent, will lose water rapidly and soon become practically completely dry. When a proper amount of the granules or powdered material is added to a non-aqueous or organic liquid containing water, as for example ordinary 95% ethyl alcohol, an instantaneous rise in temperature is noted and upon filtration or distillation the liquid is found to be practically free from water.

On the other hand, when the heating of the dihydrate or half-hydrate is carried to temperature above about 300° C. for considerable periods of time, the product no longer possesses a strong affinity for water. Such a product rehydrates more slowly or not at all, and is accordingly useless as a drying or desiccating agent.

The rate of dehydration varies directly with the temperature. For example, when heated in ordinary air the dihydrate begins to lose water very slowly at slightly above 100° C., while the dehydration goes on actively only at temperatures approximating 160° to 170° C. and proceeds at a rate which is economically satisfactory at 230° to 250° C. Temperatures up to slightly above 300° C. may be used, but with increasing liability of overheating.

In practice I have found that when either the powdered or granular dihydrate is heated at 230° to 250° C. for about two hours the product obtained is practically completely dehydrated and has a very strong affinity for water, thus making it an excellent drying or desiccating agent. This heat treatment may be carried out by spreading the granules or powder in uniform layers, preferably ½ inch to 2 inches deep, in iron or other suitable pans or trays and heating in a gas or electric oven or other suitable oven or heating device.

The action of dehydration of the calcium sulphate dihydrate may be greatly expedited by passing a current of dry air over the material or by passing a current of moist air heated to such a degree that its vapor content is lower than the saturation point, so that it is still capable of receiving moisture from the calcium sulphate rather than passing moisture to it.

Since the anhydrous calcium sulphate prepared as described above has a very strong affinity for water either in the liquid or vapor form, the freshly prepared material must be protected from exposure to moist air. The product will not combine with water above, say, 200° C. It has therefore been found best to place the freshly dehydrated material in strong sealed containers while still at a temperature of approximately 200° C. I have found it desirable in handling large batches to place the hot material in vessels provided with inlet tubes through which dry air may be admitted as the material cools and the surrounding air contracts. When cooled, the material may be transferred rapidly and with proper precautions to suitable air-tight containers, as bottles, cans or drums, in which it may be stored indefinitely for use as required.

The drying agent prepared as described above has a capacity for combining chemically with approximately 6.6 per cent of its weight of water while acting efficiently as a drying agent. Since the product formed by the absorption of this amount of water is a relatively stable hydrate, namely, the half-hydrate, the rate of absorption and the drying efficiency of the drying agent is maintained uniform until practically this amount of water is absorbed. When this amount of water has been absorbed, the drying agent becomes inactive and ineffective. However, by reheating exactly as in the original preparation, namely, for about two hours at about 230° to 250° C., the material is entirely regenerated and its drying efficiency and capacity are restored. This regeneration of the exhausted drying agent may be repeated indefinitely without noticeable loss in drying efficiency or appreciable alteration of its granular form or strength.

What I claim is:

1. The process of manufacturing a drying agent which consists in reducing calcium sulphate dihydrate to calcium sulphate half-hydrate, and subsequently heating the half-hydrate at a temperature less than 300° C. to form a highly porous calcium sulphate substantially free from water of hydration.

2. The process of manufacturing a drying agent which consists in heating hydrated calcium sulphate at approximately 230° to 250° C. for a length of time sufficient to effect practically complete dehydration.

3. The process of manufacturing a drying agent which consists in heating hydrated calcium sulphate in granular form for about two hours at a temperature between 230° C. and 250° C. to effect practically complete dehydration and produce a porous product without materially altering the size or form of the granules.

4. As a new drying agent, substantially completely dehydrated calcium sulphate having an affinity for water greater than that of ethyl alcohol.

5. As a new drying agent, granules of substantially completely dehydrated calcium sulphate having an affinity for water greater than that of ethyl alcohol.

In testimony whereof I have hereunto signed my name to this specification.

WILLIAM A. HAMMOND.

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From DEFENDANT'S EXHIBIT 1.

Aug. 21, 1934. **W. J. ELSER** 1,970,956

METHOD OF DESICCATING LIQUIDS AND SEMISOLIDS

Filed May 16, 1931 3 Sheets-Sheet 3

Fig. 8.

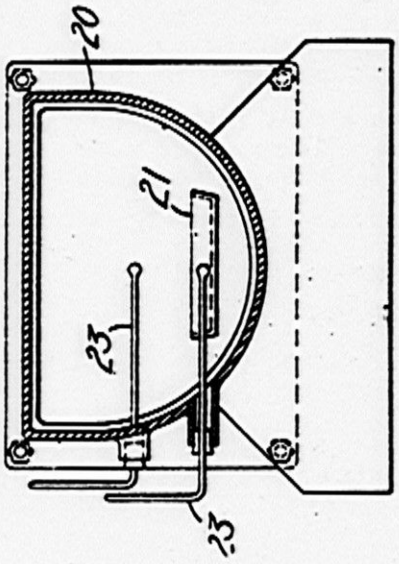


Fig. 9.

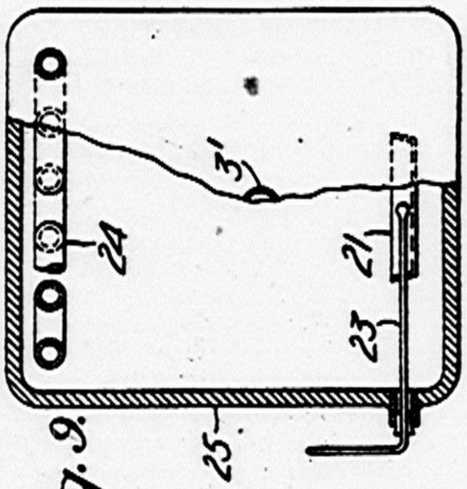


Fig. 7.

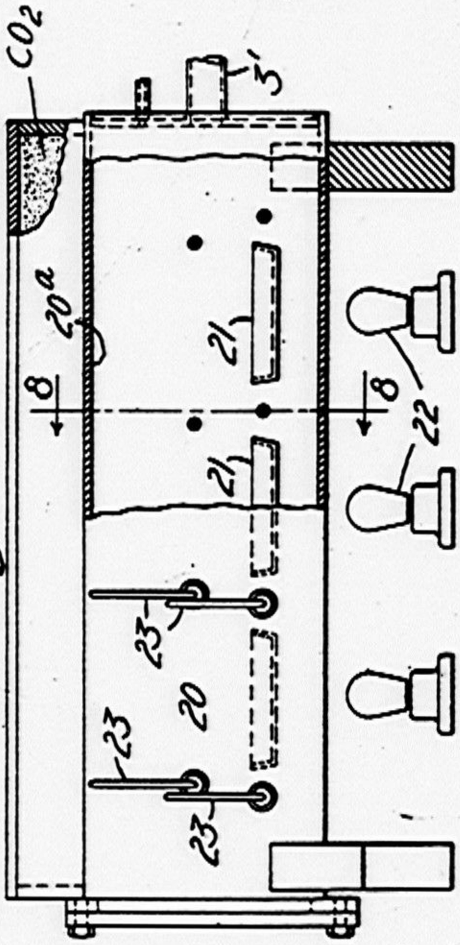
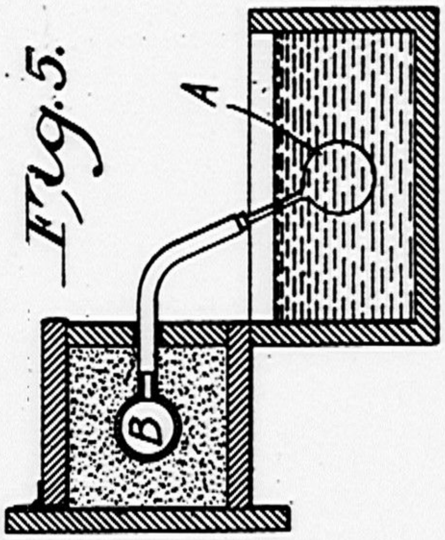


Fig. 5.



Inventor

William J. Elser

By Attorney

Nathan, Bowman & Helferich

**Aug. 21, 1934.** W. J. ELSER **1,970,956**

METHOD OF DESICCATING LIQUIDS AND SEMISOLIDS

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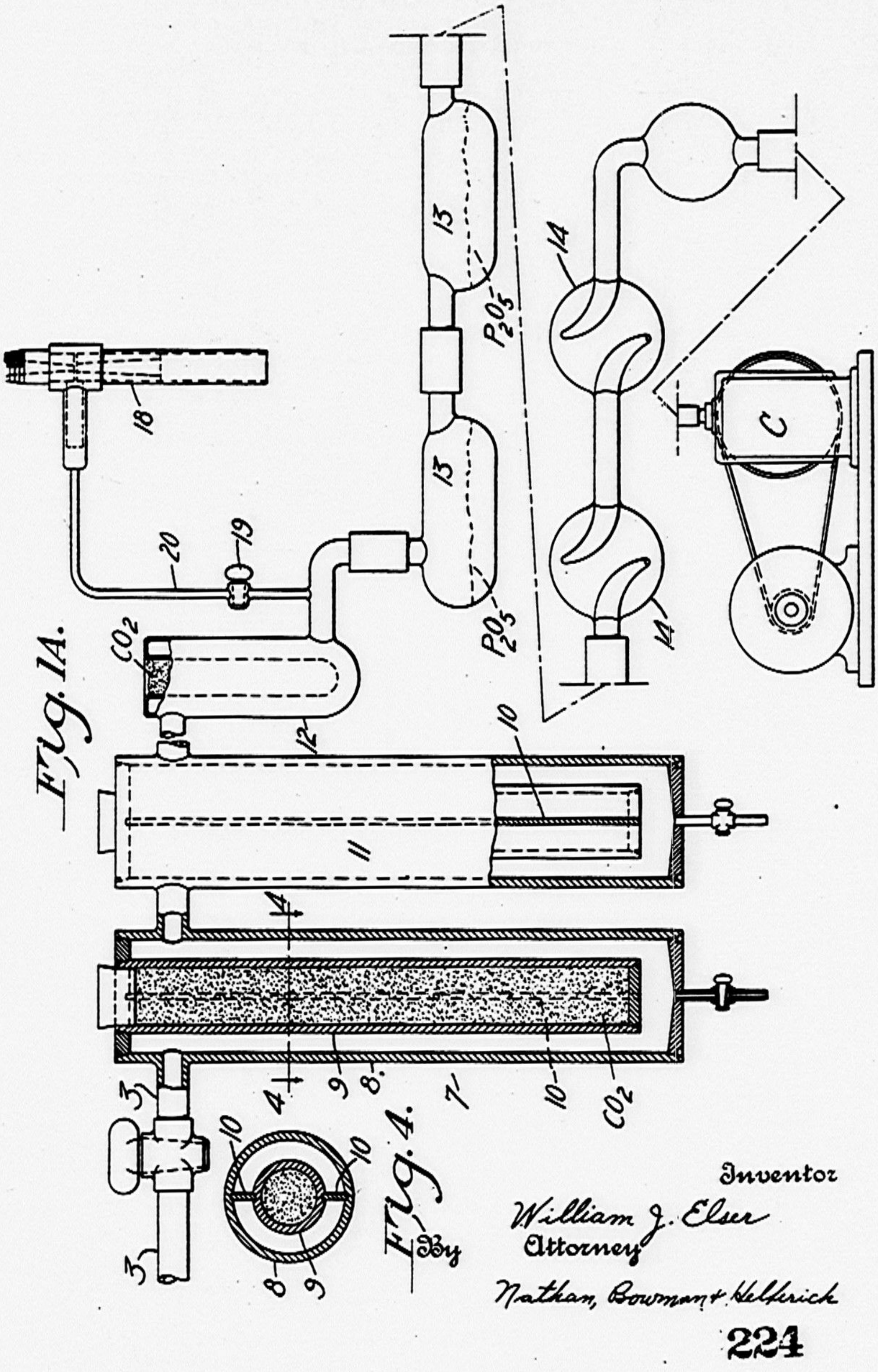

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**Aug. 21, 1934.** **W. J. ELSER** **1,970,956**

METHOD OF DESICCATING LIQUIDS AND SEMISOLIDS

Filed May 16, 1931 3 Sheets-Sheet 1

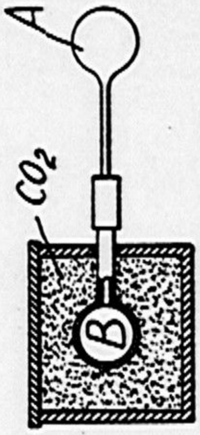

Fig.6.

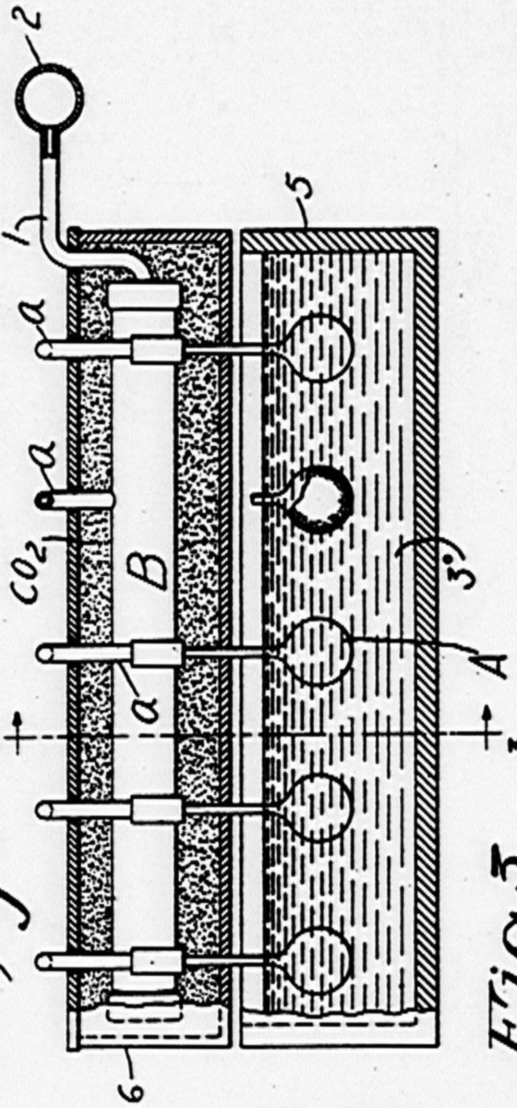

Fig.2.

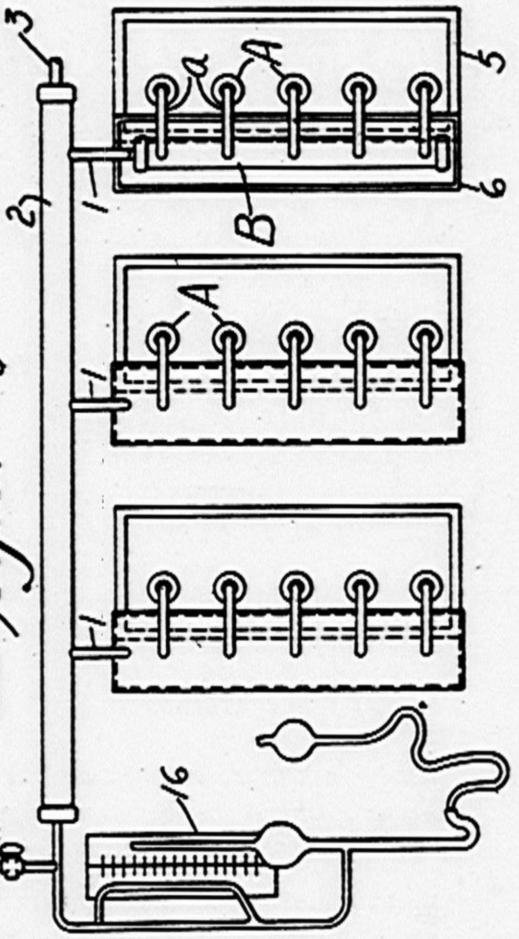

Fig.5.

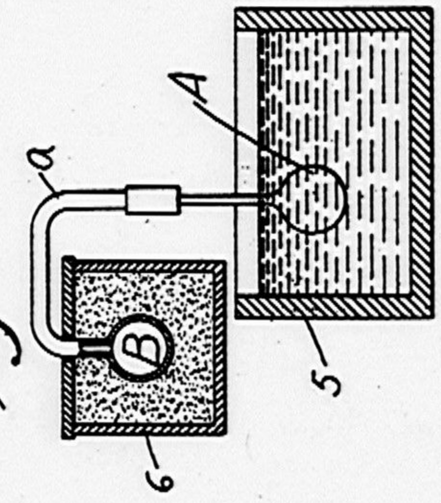

Fig.1.

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**Patented Aug. 21, 1934** **1,970,956**

# UNITED STATES PATENT OFFICE

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1,970,956

## METHOD OF DESICCATING LIQUIDS AND SEMISOLIDS

William J. Elser, Old Greenwich, Conn.

Application May 16, 1931, Serial No. 537,898

8 Claims. (Cl. 99—5)

This invention is concerned with the proposition of so-conditioning liquids and semi-solid materials containing proteins or other biological derivatives that, without alteration in their essential properties, they may be preserved for future use in unaltered form. To carry out this conception, this invention, through the agency of suitable apparatus, subjects the material to a method of treatment which converts it into a permanent product capable of being, at any subsequent time, restored to its original form and equally available for immediate usage.

If the water constituent and gases of blood-sera and semi-solid materials could be eliminated without altering their other characteristics, or introducing any foreign substance, it was apprehended that the thus-desiccated product would not be subject to deterioration, and by the mere addition of pure water, could be used in lieu of the original fresh material for all purposes. This expectation has been realized as herein explained.

It has likewise been established that if, prior to and during the operation of withdrawing the water from the material, such water be segregated from the tissues and cells so far as possible, then can its evaporation occur without deterioration. To this end, the material is, as a preliminary step, chilled below the freezing point of water; the chilling being performed expeditiously with the object of segregating the water in the form of crystals as minute as possible. If this condition be maintained until the water has completely been removed by evaporation of these ice crystals, and if the evaporation be caused to take place expeditiously, it is certain that no deterioration will result.

Inasmuch as the vapor pressure of water below its freezing point is exceedingly low (4.58 m.m. Hg. at 0° C.; 4.22 at −1° C.; 3.88 at −2° C.; 3.57 at −3° C. and 3.28 at −4° C.) evaporation requires to be carried out through specially contrived means and under particular conditions.

This admits of being set forth to better advantage through reference to the annexed drawings in which:—

Figures 1 and 1A combined represent one form of apparatus well adapted to carry out the desiccation of quantities of sera in accordance with this invention. Fig. 2 is a longitudinal sectional view through one of the desiccating units illustrated in Fig. 1. Fig. 3 is a sectional view taken along line 3—3 of Fig. 2. Fig. 4 represents a sectional view through line 4—4 of one of the vapor traps illustrated in Fig. 1A. Fig. 5 is a modified form of the connections between the sera containers and the condensing chambers. Fig. 6 is a similar connection adaptable however for cold storage room purposes. Fig. 7 represents an improved form condensing chamber within which are placed trays of the material to be desiccated. Fig. 8 is a sectional view through line 8—8 of Fig. 7. Fig. 9 is a further form of condensing chamber and which is cooled by means of a refrigerating coil instead of $CO_2$ snow or other refrigerant as in Figs. 2 or 7.

The primary elements of the apparatus comprise a container A for the serum, a condensing chamber B for the collection of the water withdrawn from the serum, and a pump C for withdrawing all initial air or other gases from the system and for maintaining it under a high vacuum.

It may here be noted that this system neither depends upon nor contemplates chemical absorbents for performing the desiccation. Although such agents, as for example, phosphorus pentoxide have a powerful affinity for water, and a very low vapor pressure, nevertheless they (or perhaps, certain impurities thereof) are volatile to the extent of their vapor pressures, and may bring about changes in the serum such as to defeat the purpose of the invention. This has been demonstrated to be a fact.

It is, therefore, proposed to avoid such absorbents and to accomplish the desiccation by employing a condensing chamber, the inner surfaces of which are maintained at a temperature so low that the vapor pressure of water (ice) in contact therewith will be materially lower than ice at 0° C. or a few degrees lower. To illustrate, (assuming the pump is capable of producing a complete vacuum) if the condenser be surrounded by $CO_2$ snow its wall will be cooled to a temperature of about −70° C. At this temperature, ice has a vapor pressure of only about .0025 and hence the water vapor evolved by the frozen serum (under a vapor pressure of about 3.0 m. m. Hg.) will be caused to condense to the extent of maintaining in the space a vapor saturation of a very low order; to wit, only such as corresponds to a pressure of .0025 m. m. Hg. If instead of solid $CO_2$, there be used liquid air to cool the condenser, the differential in vapor pressure will be even greater; to wit less than $6.6 \times 10^{-16}$ m. m. Hg.

In the interest of rapidity of evaporation of the water from the serum and of its speedy accumulation as ice in the condenser, it is desirable to approach the foregoing ideal condition as nearly as possible. This pre-supposes a pump capable of removing all of the initial air and gases from the system. While no pump can create a perfect vacuum, pumps are available which will exhaust the system to as low a pressure as 0.007 m. m. Hg. (or lower). By resorting to such a pump, in combination with the system as described, it has been found to be possible to dehydrate sera effectively.

Considering the case from the standpoint of the minimum pressure creatable by the pump (say 0.007 m. m. Hg.) the condenser should be maintained at a temperature at which the vapor pressure of water (ice) is less than 0.007. At a temperature of minus 61 degrees C., the vapor pressure of water is 0.007; and the condenser should be kept below this point under these conditions. So long as the pressure in the system is lower than about 3.0 m. m. Hg., the frozen water in the serum will be above what may be said to be its boiling point and hence will evaporate speedily.

Certain practical considerations are likewise noteworthy. The material should be arranged to present a relatively large surface, and the condensing area should be no more remote than need be from the material; for water-vapor extends but slowly in a vacuum. The first condition may be attained easily, as a preparatory step, by rapidly revolving the bulb containing it, while held in a slanting position, in a freezing bath such as an ice-salt mixture at between —12° and —20° C. This will interiorly coat the bulb with a film of the frozen material. The second condition relates to the arrangement of the apparatus. This may now be described through detailed reference to the annexed drawings.

Referring more particularly to Figs. 1 and 2, it will be observed that a series of bulbs or containers A are illustrated as connected by short tubes *a* to a common condensing chamber B which is connected by means of tubes 1, 2 and 3 to the remainder of the drying apparatus illustrated in Fig. 1A. The bulbs containing the frozen mixture to be desiccated are maintained at a temperature slightly below zero by being submerged in a suitable coolant contained in a tank or vessel 5, or if desired and if local conditions permit the bulbs may be arranged to extend laterally from the condensing chamber B as in Fig. 6. This last mentioned arrangement is particularly adaptable for cold storage room purposes, that is, in such cases wherein the apparatus is set up in a room or chamber in which the temperature is maintained constant and at approximately —3°, thereby dispensing with the baths 5.

The condensing chamber B is, in this figure, enclosed in a box 6, filled with $CO_2$ snow which cools the walls of the chamber B to a temperature approximately —70° C. whereby the vapors rising from the bulbs A will immediately condense and solidify on the interior thereof. It may be here stated that the interior surface area of the condensing chamber is so proportioned with respect to the volume of sera to be dried that the accumulation of ice rapidly formed therein is deposited in a thin layer more or less evenly over the entire inner surface.

The air is then withdrawn from the condensing chamber and evaporation of the sera takes place. One end of the chamber B is connected to a high vacuum pump C and in order that no moisture is carried to the pump, the air is first passed through a series of traps and dryers now to be explained. Referring to Fig. 1A, it will be seen that the exhaust line 3 connects with a vapor trap 7 which comprises two tubes 8 and 9, one within the other, which are separated longitudinally by two baffle plates 10 arranged diametrically opposite each other, as shown in Fig. 4. The inner tube 9 and baffle plates 10 are placed a short distance from the bottom of the outer tube 8, thereby forming a U shaped chamber, as shown in Fig. 1A, for the passage of the exhaust air. The inner tube 9 is cooled by filling same with $CO_2$ snow and as the exhaust air comes into contact therewith a rapid chilling takes place which removes moisture that has passed the main condensing chamber B. This operation is again repeated by passing the air through a second vapor trap 11, constructed in all respects identical with the trap 7.

The pump is still further safe guarded against sucking moisture laden air by the insertion of a third vapor trap 12, which in principle operates as the traps 7 and 11, but is made of transparent glass so that whatever moisture is condensed therein is apparent to the observer as a frosty ice deposit. The inner tube of the indicator trap is painted black so as to increase the visibility of the minute particles of ice that may form therein.

Before the withdrawn air reaches the pump it passes through one or more drying tubes or chambers 13 which are partly filled with a water vapor absorbing powder such as phosphorous pentoxide ($P_2O_5$) which takes up the last vestige of moisture that may still be carried by the air as it is withdrawn by the pump. Dust traps 14 collect and retain the particles of $P_2O_5$ powder that may be picked up and carried to the pump when the powder is perfectly dry and when the volume of air and the rate of flow is great as is the case during the early periods of evacuation. This may be more clearly understood if it will be remembered that when the apparatus is first set up and ready for operation, the pump, in withdrawing the air, gases, etc., in large quantities.

The pumping operation is continued until the desired degree of vacuum (say .007 m. m. Hg.) is reached, and then shut off.

If there is no leakage of air into the system after the vacuum is attained the drying process continues rapidly and uninterruptedly until the product is thoroughly dried. However, as a practicable problem, a high vacuum is difficult to maintain and, therefore, it is preferable that the pump be left connected with the system and operated intermittently or continuously to maintain the vacuum so that the drying process will not be retarded.

For the purpose of keeping a record of the degree of vacuum maintained when drying a given mixture an indicator or gauge such as the conventional MacLeod gauge 16 is attached to the system preferably at a point remote from the pump. Accurate readings may then be taken and comparative records made of various mixtures undergoing desiccation.

Upon completion of a drying operation bulbs are removed and corked. The apparatus is then quickly and easily "defrosted" by opening the cock 17 which connects the tube 1 with a source of dry air that is drawn through the condensing chamber and vapor traps by suitable means, such as, for example, a water pump 18. A stop cock 19 placed in the conduit 20 leading to the pump effectively closes this line when the pump C is operating to evacuate the system.

It is obvious that various other forms and arrangements of condensing chambers may be resorted to without departing from the principle of operation underlying this invention. Fig. 5 illustrates a modified form of the connection between the bulbs A and cooling chamber B in order to reduce the distance that vapor must travel before being condensed. It may be here stated that the chamber B may be connected directly to the traps and pump previously explained and that any number of the containers A may be connected therewith according to the amount or quantity of mixture that is desired to be dried.

Larger quantities of the mixture may effectively be dried by placing it in pans or trays inserted within a vacuum tank or chamber in which the differential in temperatures are maintained. Such an apparatus is depicted by Figs. 7 and 8 in which 20 indicates an air tight chamber in which the trays 21 are placed and supported a short distance from the bottom thereof.

The top of the drying chamber is covered with $CO_2$ snow, held in place by a wood frame, which chills the under surface $20^a$ thereof to approximately —70°.

The product to be dried is placed in the pans 21 within the drying chamber and the latter connected to the pumping apparatus by means of the conduit 3' and the entire system evacuated. As evacuation proceeds the gases contained in the product are liberated and escape with the air and when the vacuum therein reaches a certain value, boiling of the liquid occurs which is followed by freezing brought about as the result of rapid evaporation.

The liberated vapors when coming into contact with the chilled surface of the drying chamber immediately condense and freeze thereon, practically none of the vapor passing to the $CO_2$ traps 7, 11 and 12 and in this manner the material whether in liquid form or in solid form or whether it be serum or any other semi-solid containing protein that is placed in the pans 21 is quickly and easily desiccated.

So that the temperature of the product does not reach a value so low as to impede vaporization of the liquid heating means may be provided, such as the lamps 22, for maintaining a given temperature in the lower areas of the drying chamber 20 to counteract the cold transmitted to the product by the chilled top surface of the tank, and the cold caused by the evaporation of the liquids. A series of thermometers 23 placed in the chamber indicate the temperature existing in the immediate vicinity of the product to be dried and at other sections of the chamber so that the heating elements 22 may be regulated accordingly.

Similarly, the degree of chill given to the top surface may be regulated by the use of a refrigerating coil 24 placed near the surface thereof and which may be controlled in the conventional manner. Fig. 9 illustrates a drying chamber 25 in which the refrigerating coils have been placed and which in other respects is similar in operation to the apparatus illustrated in Figs. 7 and 8.

Rapid evaporation of the liquids and the speedy accumulation of ice is still further accelerated if the product to be dried is first charged with a gas, such as $CO_2$ gas (because of the relatively large quantity thereof that can be absorbed in water) and the aerated product placed in the bulbs or trays and dried in the manner previously explained.

Presenting the material in a frothy state hastens the drying process because of the greater number of areas that are exposed or subjected to the differential temperatures in the vacuum and, as a consequence, vaporization of the liquid takes place more quickly. Care should be taken, however, in the pre-charging of the product so that only enough gas will be injected to give the material the desired degree of porosity when frozen. If the material is too highly charged with a gas, excessive frothing occurs, when a vacuum is created, to such an extent that the material escapes from the open trays. It is desirable to cause frothing, but it should not be excessive and I have found that somewhat less than 25% by volume of $CO_2$ gas for a quantity of serum 7 m. m. deep in a tray measuring 1½" x 6" x 7" will be sufficient to cause frothing of the liquid. There are certain factors, however, which must be taken into consideration when determining the amount of charge, chief among those being the efficiency of the particular apparatus employed and the rapidity of evacuation, other variables such as local temperatures, the peculiarities of a given specimen to be dried, the charging gas used, etc., must also be considered and inasmuch as local conditions govern each particular case the amount of pre-charging is usually determined by test. Suffice it to say, however, the greater the charge, the shorter will be the interval of time required in transforming semi-solids to a dry state.

The product prepared by the foregoing method, and through the agency of apparatus such as described, is in the nature of a dry powder readily soluble in water or capable of absorbing water to revert to its original condition. It is quite sterile and remains so for a long period of time, if not indefinitely, if not exposed to unfiltered air. So far as I have been able to determine, through many delicate tests applied to a great variety of different immune and normal sera as well as by the use of different sensitive indicators, the product is not altered in its initial properties and it will remain unalterable in this state; certainly if kept in a vacuum in an ordinary refrigerator.

In view of the importance of producing sera unaltered and unalterable with absolute certainty, I have, in disclosing my discoveries, set forth all such conditions and steps as have definitely resulted in success. Some of these, admit of modification or omission in going from the general case to certain specific instances.

Where, for example, an absolute sterility is not essential in the product, the procedure and apparatus is capable of simplification and the quantities that can be handled may be increased by 100 or even 1000 fold, and even such large quantities can be produced sterile by adapting sterile precautions. Thus, chilling by conventional refrigerating means may be resorted to; in other specific instances, the preliminary step of conditioning the material by quickly freezing it, ab initio, need not be imperative; in still other specific instances, the period of desiccation of the chilled material may be materially extended and a smaller differential in vapor pressure employed without causing deterioration of the product. Whether such variations may safely be resorted to must needs be contingent upon the material undergoing desiccation and the use for which it is destined.

It is a fact, for illustration, that very gradual freezing, as it takes place in nature, results in deterioration of certain immune sera, and for that reason rapid freezing was tried, found to be successful, and accordingly prescribed as the best mode now known to me for putting my invention into effect.

Believing that the discoveries and invention herein disclosed may open a field of useful research, I hereby disclaim any intention to restrict any original and experimental work of any kind, and hereby dedicate to the public the use of the same for all such purposes; all, however, with the express reservation and understanding that their usage for all commercial purposes shall remain entirely under my control and direction.

I, therefore, claim:

1. The method of rendering sera immune to deterioration and effectively available for future use which consists in exposing to a temperature approximately zero degrees centigrade the serum and in vacuo subjecting the water vapor emitted thereby and at a space therefrom to a temperature much lower than 0° C. whereby the serum will be desiccated to a dry powder capable of being restored to its original condition upon the addition of water.

2. The art of desiccating a serum into a stable product which consists in exposing it in filmlike form and while maintained at a temperature approximately one or two degrees below zero to a vapor-space under a reduced pressure, and simultaneously maintaining a portion of said vapor-space at a temperature lower than zero degrees centigrade to condense, in the form of ice, all water-vapor given off by the serum.

3. A process of desiccating a serum without biological alteration into a stable form which consists in maintaining it in a closed chamber at a temperature relatively less than 0° C. and simultaneously maintaining a communicating receptacle at a temperature of about −70° C. or lower for a sufficient period of time to enable all of the water content of the serum to be transported to and retained in frozen form by the said remote wall of said chamber, said closed chamber and receptacle being maintained at a lower pressure than that of the vapor pressure of water.

4. The method of transforming without deterioration semi-solids containing protein to a dry state available for future use which consists in exposing to a temperature approximately zero degrees centigrade the water content of a given semi-solid and in a vacuum and subjecting the water vapor emitted thereby and at a space therefrom to a temperature lower than said first mentioned temperature.

5. The method of desiccating semi-solids containing protein without deterioration which consists in vaporizing the water content of a given semi-solid at a temperature approximately zero degrees centigrade and subjecting the water vapor emitted to a temperature lower than said approximate zero degree centigrade and at a point removed from the material undergoing desiccation.

6. The method of transforming without deterioration semi-solids containing protein to a dry state which consists in charging the semi-solid with a gas, to a temperature approximately zero degrees centigrade the water content of the charged semi-solid in a vacuum, and subjecting the water vapor emitted thereby, and at a space therefrom, to a temperature lower than said first mentioned temperature.

7. The method of desiccating semi-solids containing protein without deterioration which consists in charging the semi-solid with a vaporizing accelerator, vaporizing the water content of the charged semi-solid at a temperature approximately zero degrees centigrade and subjecting the water vapor emitted to a temperature lower than said first mentioned temperature and at a point removed from the material undergoing desiccation.

8. The method of converting semi-solids containing protein to a dry state without deterioration which consists in aerating the semi-solid material by charging the said material with a gas, under a given pressure, subjecting it to a lower pressure whereupon the semi-solid charged material is caused to expand in aerated form; exposing the said material while in that form to a temperature approximating two degrees below zero centigrade, and then vaporizing the water content thereof and collecting same at a place removed from the material undergoing dessication.

WILLIAM J. ELSER.

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## CERTIFICATE OF CORRECTION.

Patent No. 1,970,956. August 21, 1934.

WILLIAM J. ELSER.

It is hereby certified that error appears in the printed specification of the above numbered patent requiring correction as follows: Page 4, line 94, claim 6, after "gas," insert the word exposing; and that the said Letters Patent should be read with this correction therein that the same may conform to the record of the case in the Patent Office.

Signed and sealed this 30th day of October, A. D. 1934.

Leslie Frazer
Acting Commissioner of Patents.

(Seal)

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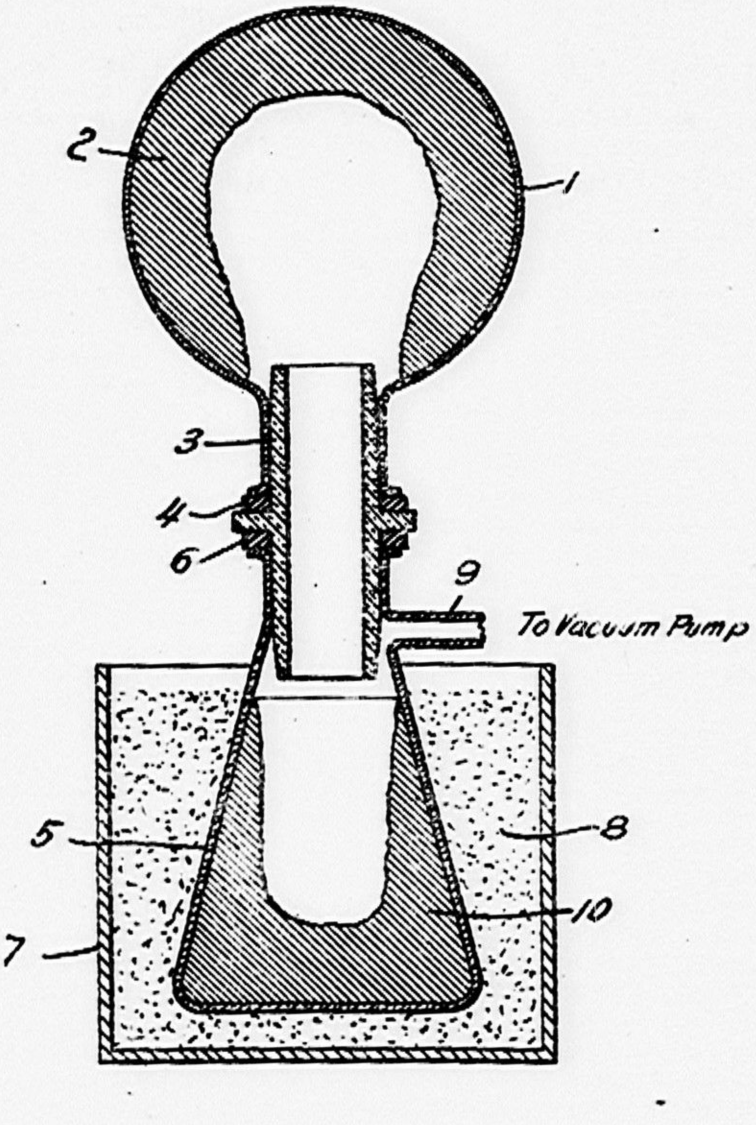
From DEFENDANT'S EXHIBIT 1.

**Dec. 29, 1936.** J. REICHEL **2,066,302**

LYOPHILIC BIOLOGICALLY ACTIVE SUBSTANCES AND PROCESS OF PRODUCING THE SAME

Filed Jan. 13, 1934



John Reichel *INVENTOR*

BY Pennie Davis Marvin Edmonds

*ATTORNEYS*

**Patented Dec. 29, 1936** **2,066,302**

# UNITED STATES PATENT OFFICE

2,066,302

## LYOPHILIC BIOLOGICALLY ACTIVE SUBSTANCES, AND PROCESS OF PRODUCING THE SAME

John Reichel, Philadelphia, Pa., assignor to Sharp & Dohme, Incorporated, Philadelphia, Pa., a corporation of Maryland (1929)

REISSUED

Application January 13, 1934, Serial No. 706,547

11 Claims. (Cl. 99—199)

This invention relates to new lyophilic biologically active substances, and includes both the new substances and products and a method of producing them. More particularly the invention relates to new lyophilic sera and other biologically active substances which are prepared in the lyophilic state by the removal of water therefrom so that the lyophilic product, on being restored to the liquid state by the addition of water, will correspond closely in biological or therapeutic activities with the sera or other products in a fresh state.

Substances known as biological products, being biologically active or possessing immunizing or therapeutic value, such as sera, are used in the diagnosis, prevention and treatment of diseases of man and animals. In the form in which they are generally available at the present time they are unstable and deteriorate under the influence of physical and chemical agencies such as heat, preservatives, enzyme action, oxidation-reduction phenomena, hydrolysis, and other intramolecular changes.

Since the potency efficiency or biological activity of the substance is effected by such changes, and since such changes take place more readily in the presence of water, that is, when the colloids are dispersed in water, attempts have been made in the past, especially in the case of sera, to prevent such changes or deterioration by evaporating or removing the water from the substances at temperatures near and above the freezing point of water; but the dessication of such substances near and above the freezing point of water, that is, drying from the liquid or partial liquid state, has not given the desired results.

In the case of sera, the removal of the water by these methods brings factors into play such as formation of surface skins and gradual concentration of inorganic salts which alter the physical and chemical properties of the substances as is evidenced by their incomplete and slow solubility in water, in the formation of particles possessing a hard, granular, gritty structure with a relatively high density and marked yellow color, and in the denaturation of the proteins. Such processed sera and other substances are not completely restored by the addition of water and the rate of the partial restoration is slow compared with lyophilic substances as processed and herein described.

Furthermore, since the biological or therapeutic activity of complex substances such as sera is extremely susceptible to physical and chemical changes, experience has shown that substances dehydrated from the liquid or partially liquid state, fail to conserve all of the original biological or therapeutic activities.

The present invention overcomes the objections to prior products, such as those above referred to and provides new and valuable biological substances and products in a lyophilic state, which, on being restored by the addition of water to the lyophilic products possess the same or practically the same biological and therapeutic activities originally possessed by the substance in the so-called fresh state. The new lyophilic products, when properly produced, and protected from deterioration, will be practically identical with the original fresh material both physically and biologically.

According to the present invention, the serum or other biological product is transformed from the liquid to the completely frozen solid state by substantially instantaneous freezing of the liquid by indirect contact with a freezing agent such as liquid air, dry ice (solidified carbon dioxide) or other low temperature refrigerant or freezing mixture, and by subliming the ice from the frozen mixture while preventing melting thereof, while maintaining a high vacuum, the frozen water being removed from the frozen material without melting or softening the material.

The substantially instantaneous freezing of the serum or other liquid biologically active product is advantageously carried out by placing the liquid material in a container, such as a spherical glass flask or metal cylinder and then freezing the material quickly, solidly and completely on the inside of the container, preferably in the form of a thin layer on the inner surface of the container, by immersing the container in a freezing mixture at a temperature far below the freezing point of the material or by spraying the charged container with the cooling substance, using such cooling substances as liquid air, dry ice, or other freezing mixture at a temperature far below the freezing point of the material.

In order to insure quick and substantially instantaneous and complete freezing it may be necessary or desirable to add the liquid material to the chamber or container by installments and to allow the material to freeze solidly in the form of layers on the inside of the chamber. When a layer of the material is thus frozen in a substantially instantaneous manner to a temperature far below the freezing point, the addition of a further amount of the material will result in its substantially instantaneous freezing by coming in contact with the already frozen layer of material at such a low temperature, and by the further cooling action of the liquid air or other refrigerant in contact with the outside of the container.

When the chamber or container has become charged to the desired extent with the completely and solidly frozen serum, etc., which is frozen in a substantially instantaneous manner, the charged chamber or container is then connected to a condenser which is packed in or embedded in a freezing mixture at a sufficiently low temperature and which is also connected with a high vacuum pump, and the air is exhausted and a high vacuum maintained to effect sublimation or removal of the ice from the material in the chamber or container at a temperature below that at which the material melts or becomes liquid. The condenser may, for example, be covered or packed with or embedded in a freezing mixture such as dry ice, or a mixture of acetone and dry ice, or liquid air. The vacuum employed is as nearly a perfect vacuum as is readily obtainable with a high vacuum or super-vacuum pump. When the vacuum becomes sufficiently high, water vapor leaves the frozen charge, without melting the charge, and is collected in the form of ice in the condenser. In order to increase the rate of evaporation of the water in the frozen material, where this material is at too low a temperature, the charged chamber may be warmed with warm circulating air or by immersing it in a warm liquid, but the heat applied should not be sufficient to melt or even soften the frozen charge.

By maintaining a sufficiently high vacuum, and by regulated warming of the outside of the container of the solid frozen material to a temperature which still maintains the frozen material solid and without melting or softening, the ice contained in the frozen material is sublimed and removed without converting the frozen material into a liquid state and there remains, after the removal of the ice a dry product which has been freed from its frozen water while in a completely frozen state. When the frozen water has been completely or almost completely evaporated from the material, the charged chamber and its contents take on the temperature of the surrounding atmosphere, but prior to that time the charged chamber and its contents are at a temperature sufficiently below the freezing point to maintain the product in a solid frozen state while the ice is being removed by sublimation therefrom.

As a result of this treatment of the serum or similar biological product there are avoided or prevented any apparent physical or biological changes in the substance which is instantaneously frozen and lyophilized while maintained in its frozen state, and as a result there is obtained a final product which has not been deteriorated by treatment in a liquid state while the water is being removed therefrom. By transforming the liquid substance quickly and completely into a frozen solid state, the component parts of the colloidal system are permanently fixed and changes are prevented in the chemical equilibrium such as would occur at the surface in the dehydration of a liquid product, and such as frequently results in the formation of polymers or anhydrides of the protein or other constituents. It is well known that when such surface films occur, in the evaporation of aqueous materials such as sera, they possess different properties from the rest of the proteins as is shown by their marked insolubility.

The removal of the frozen water from the solid frozen material does not result in contraction of the volume of the material such as occurs when water is removed from liquid materials. As a result, the lyophilic material, after the removal of the frozen water therefrom, retains the same or practically the same volume as the frozen solid before the removal of the ice therefrom.

The rapid freezing of the serum or similar material and the removal of the frozen water from the frozen solid material leaves the organic residue of the material in a porous and sponge-like condition, with the structure of the colloidal particles apparently unchanged. The sponge-like network of fine particles forming the lyophilic material is apparently interspersed with a relatively immense network of minute capillary pores or passages from which the frozen water has been removed by sublimation, without apparently otherwise affecting the organic material in any objectionable way. When this material is added to water, or when water is added to it, the water readily and rapidly penetrates the material and reconverts it into a condition similar to that of the fresh serum or other liquid biological products before treatment. The material is also restored with substantial completeness to its original state or to a state similar to its original state without the presence of insoluble products such as are present in evaporated liquid sera, etc.

The lyophilic material is characterized by a light, porous texture. The end product is very friable and on the application of even a slight pressure it can be rubbed into a fine light-colored powder. As a result of this peculiar structure the density of the product is very low. In the case of sera products the material may have a density of about 0.13 in an unpulverized state and a density of about 0.25 after reducing to a powder. Under the microscope the pieces present a capillary, net-like structure. These characteristics impart to the products marked lyophilic properties.

The new lyophilic substances of the present invention may be brought back to a state similar to their original state by the addition of water equal in amount to that removed by the removal of water therefrom; or, where a more concentrated product is desired, by the addition of a lesser amount of water than that removed.

The new lyophilic biological substances retain their activity and value in a practically unaltered state for longer periods of time, and are less susceptible to changes due to heat and preservatives, than the original liquid preparations. The new materials, when properly prepared, will first float on the surface of water and then promptly dissolve and disappear, restoring a condition similar to that of the original serum or other material before treatment, being thus distinguished from substances dried or dehydrated in a liquid state and which usually pass down beneath the surface of the water and some of which remain intact for varying periods of time.

The present invention is applicable to the treatment of sera of various kinds, including normal, immune, or antitoxic sera, and other biological substances of similar nature used in the diagnosis and prevention or treatment of diseases of man or animals, and enables such materials to be converted into lyophilic products having important advantages in their increased stability to the effect of time, temperature and preservatives, and in their capability of being reconverted into liquid products similar to the fresh sera, etc. before treatment. Among the substances which can thus be treated and specific lyophilic products obtained are sera of various kinds, for example, normal horse serum, antitoxins such as scarlet fever, tetanus and diphtheria antitoxins, antivenin and other sera, etc.

An apparatus suitable for carrying out the present process and for producing the new lyophilic biologically active substances is illustrated in the accompanying drawing, and the practice of the process and the production of the new products will be further described in connection therewith, as an illustrative example. The drawing shows part of the apparatus in vertical section.

In the accompanying drawing the container for the liquid material is shown at 1 as a glass flask adapted to withstand the application of a high vacuum, and the material 2 is shown in a solid state contained in this receptacle. The container 1 is supported on the member 3 with a rubber gasket or other packing 4 to insure a tight seal. The member 3 is in turn supported on the container 5 which serves as a condenser, this condenser being similarly provided with a rubber gasket or packing 6 to insure a tight seal. The condenser 5 is shown as surrounded by a refrigerant 8 contained in a tub or container 7. The refrigerant may be a freezing mixture such as dry ice, dry ice and acetone, liquid air, etc.

The condenser is connected thru a connection 9 with a vacuum pump (not shown), this vacuum pump being a high vacuum pump or a super-vacuum pump which maintains as nearly perfect a vacuum as possible.

The container 1 may vary in size from a small flask or container to a large flask or container, for example, from a container of 1 cubic centimeter or smaller capacity to a container of as much as 50 liters capacity or greater.

In the carrying out of the process a small amount of the serum or other material may be placed in the container 1 and this container then immersed in liquid air or other refrigerant which will bring about rapid and substantially instantaneous freezing of the material and reduction of its temperature far below its freezing point. The addition of further amounts of the liquid material combined with further refrigeration will result in the forming of successive layers of rapidly frozen material until a proper charge of frozen material is contained therein. The material 2 is shown as forming a thick layer on the walls of the container such as results where successive amounts of liquid are added and the container rotated so that successive layers of the material are frozen. In a flask of 50 liters capacity the charge may be, for example, thirty liters of material which is thus frozen to a solid state in the manner above described. In a flask of 20 inches diameter the layer of frozen material may be for example about 4 inches in thickness on the inside wall of the container.

When the container has thus been charged with the frozen material, which is frozen to a temperature far below the normal freezing temperature, it is connected with the condenser and the air is exhausted and a high vacuum applied. When the vacuum becomes sufficiently high water vapor leaves the frozen charge, without melting of softening of the charge and is collected in the form of a layer of ice 10 on the walls of the condenser. The rate of evaporation of the ice from the frozen material may be increased by warming the container, where it is at too low a temperature, by circulating warm air over it or by immersing it in a warm liquid, but the heat applied should never be sufficient to melt or even soften the frozen charge. The material is maintained in a solid frozen state and at a low temperature until the ice has been evaporated or sublimed therefrom when the container and its charge gradually takes on the temperature of its surroundings, this continuation of the application of the high vacuum until the substance attains a temperature substantially above 0° C. having the important advantage of promoting the removal of residual water still present in the product after the ice has been sublimed therefrom and giving a final product of improved stability, keeping properties and resistance to deterioration.

I have found it important to maintain a large and free opening or communication between the container of the frozen material and the condenser to permit the ready passage of the water vapor to the condenser. If the passage is restricted and free flow of water vapor retarded there is danger of partial or complete melting of the frozen charge, and this should be avoided.

The rapid freezing of the serum or other material retains the structure of the colloid particles and constituents and prevents change in concentration or degree of dispersion in water such as would occur in the evaporation of the materials in a liquid state. Apparently the rapid freezing of the liquid material results in a substantially complete and permanent fixing of the constituents, including colloidal constituents and inorganic salts, in the state and relative relations in which they exist in the fresh liquid material, such that, when the resulting lyophilic products are subsequently treated with fresh water they reassume practically their original state and reproduce a serum or other liquid biological product having the same or practically the same physical and biological properties as the fresh material.

It will also be noted that the material is protected, during the removal of water therefrom, while in a solid frozen state, from contact with the atmosphere, and from contact with or use of any of the known dessicating materials. The material is also protected from bacterial and mold contamination and, when treated in a sterilized condition, maintains its sterility, so that a dry, solid, lyophilic sterilized product is directly obtained.

The new lyophilic products can be kept in a lyophilic state for prolonged periods of time, for example, by sealing them in evacuated containers. The present invention therefore makes possible the preparation of products of improved keeping properties which can be stored and shipped and used when needed by the addition of a suitable amount of water thereto to reproduce a liquid product similar to the original fresh liquid serum, etc. A flask containing a mass of the lyophilic material and sealed in an evacuated state can be kept and shipped and used when desired by the addition of a suitable amount of water to reproduce the serum or other product for use. By the addition of less water than that originally removed from the fresh serum or other material a more concentrated serum, etc. can be produced. Individual doses of the lyophilic material can be packaged in sealed ampules and used as desired by the addition of the proper amount of water for an individual application of the material.

I claim:

1. As new products, lyophilic sera and other biological substances in a solid state, obtained by the rapid freezing of the liquid substances by indirect exposure to a refrigerant maintained at a temperature of about —70° C. or colder and removal of frozen water from the frozen material under a high vacuum, said substances being porous, sponge-like substances, pieces of which under the microscope present a capillary net-like structure and adapted, when water is added thereto, to form a liquid product similar to the original substance in its biological and therapeutic properties.

2. As new products, lyophilic sera and other biological substances in a porous form or in the form of a powder, said substance possessing a sponge-like or net-like structure and having marked lyophilic properties and being free from preservatives, said porous product or powder being obtained by removing the original water from the substance after freezing said substance rapidly by indirect exposure to a refrigerant maintained at a temperature of about —70° C. or colder while in a frozen state and under vacuum without having been brought in contact with or exposed to any other substance, and said substance readily reproducing a liquid, by the addition of water, having substantially the same properties as the original liquid substance.

3. The method of preparing lyophilic, biologically active substances from sera, etc. which comprises rapidly freezing the liquid material to a solid frozen state by indirect exposure to a refrigerant maintained at a temperature of about —70° C. or colder and removing the water from said frozen material by the application of a vacuum thereto while maintaining the material frozen and without melting or softening thereof.

4. The process according to claim 3 in which the water is removed from the frozen material without allowing the frozen material to be exposed to or come in contact with any other substance.

5. The process of obtaining lyophilic sera and other biological substances which comprises quickly freezing such substances in a container and removing water from the resulting solidly frozen substance under a vacuum sufficient to remove water vapor from the frozen material without softening or melting thereof while exposing the container to the atmosphere or to a bath maintained at a temperature substantially above 0° C. and with refrigeration of the escaping water vapor sufficient to form ice therefrom.

6. The method of obtaining lyophilic sera and other biological substances which comprises quickly freezing the same to a solid frozen state in a container, removing water from the solid frozen material under a high vacuum, condensing the water vapor removed from the material in the form of ice and applying sufficient heat to the solid frozen material during the removal of water therefrom by exposure of the container to the atmosphere or to a bath maintained at a temperature substantially above 0° C. to hasten the evaporation of water without melting or softening the frozen material.

7. The method of obtaining lyophilic sera and other biological substances which comprises subjecting thin layers of said substances to substantially instantaneous freezing by indirect contact with refrigerants at a temperature far below the freezing point of the substance, connecting the container of the frozen substance with a condenser through a connection providing free passage of water vapor, refrigerating the condenser with a refrigerant at a temperature far below the freezing point of water and applying a high vacuum to the container and condenser while exposing the container to the atmosphere or to a bath maintained at a temperature substantially above 0° C.

8. The method of removing water from sera and other biological products which consists in quickly freezing the substance in a container, promptly connecting the container after such freezing with a condenser, exhausting air therefrom and producing and maintaining a vacuum in said container and condenser, subjecting the condenser to refrigeration with a refrigerant such as dry ice, dry ice and acetone or liquid air, maintained at a temperature far below the freezing point of water, whereby water vapors are removed from the frozen substance into the condenser and are condensed therein into ice, and continuing the process until the substance is converted into a solid lyophilic substance by the removal of water vapor from the frozen solid and until the temperature of the substance is substantially above 0° C.

9. As new products, lyophilic sera and other biological substances in a solid state, obtained by subjecting the liquid substances to rapid freezing by exposure to a refrigerant maintained at a temperature of about —70° C. or colder, and removal of water from the solid, frozen material under a high vacuum, at a rate sufficient to maintain the substance solid, while exposing its container to the atmosphere or to a bath maintained substantially above 0° C., such vacuum being applied until the substance attains a temperature substantially above 0° C., said substance having a porous, sponge-like structure, and adapted, when water is added thereto, to form a liquid product similar to the original liquid substance in physical, biological and therapeutic properties.

10. A method of preparing lyophilic, biologically active substances from sera, etc., which comprises rapidly freezing the liquid material to a solid, frozen state by exposure of a container containing the sera, etc., to a bath maintained at a temperature of about —70° C. or colder, and removing water from said material by the application of a high vacuum thereto while maintaining the material in a solid state and while exposing the container to normal atmosphere or to a bath substantially above 0° C., whereby heat is supplied to the substance to hasten the removal of water without causing it to melt, and continuing the application of a vacuum until the substance attains a temperature substantially above 0° C.

11. The method of obtaining lyophilic sera and other biological substances which comprises subjecting such substances to substantially instantaneous freezing by indirect contact with a refrigerant maintained at a temperature below the freezing point of the substance, and removing water from said material by the application of a high vacuum thereto while maintaining the material in a solid state, and continuing the application of the high vacuum until the substance attains a temperature substantially above 0° C.

JOHN REICHEL.

From DEFENDANT'S EXHIBIT 1.

June 15, 1937. **W. E. MOORE ET AL** 2,083,732

ADSORBENT APPARATUS

Original Filed Nov. 22, 1932 4 Sheets-Sheet 4

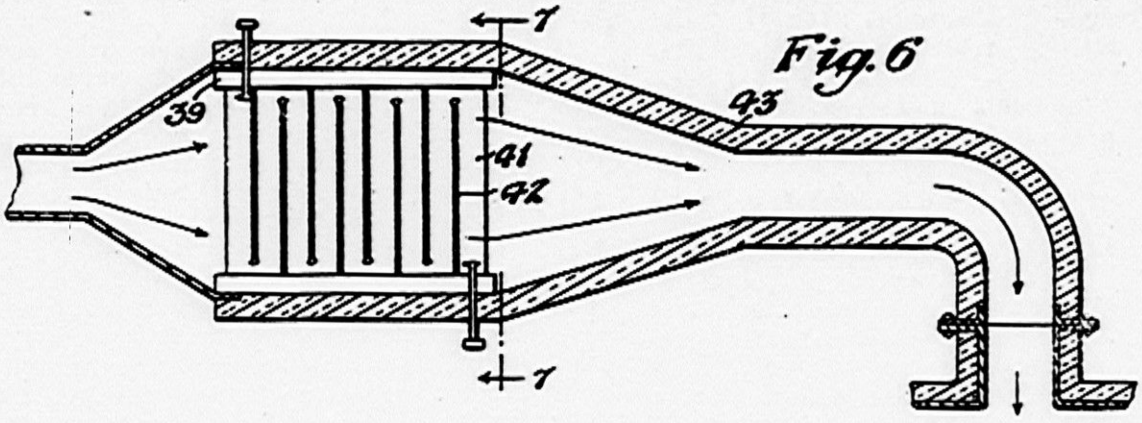


Fig. 6

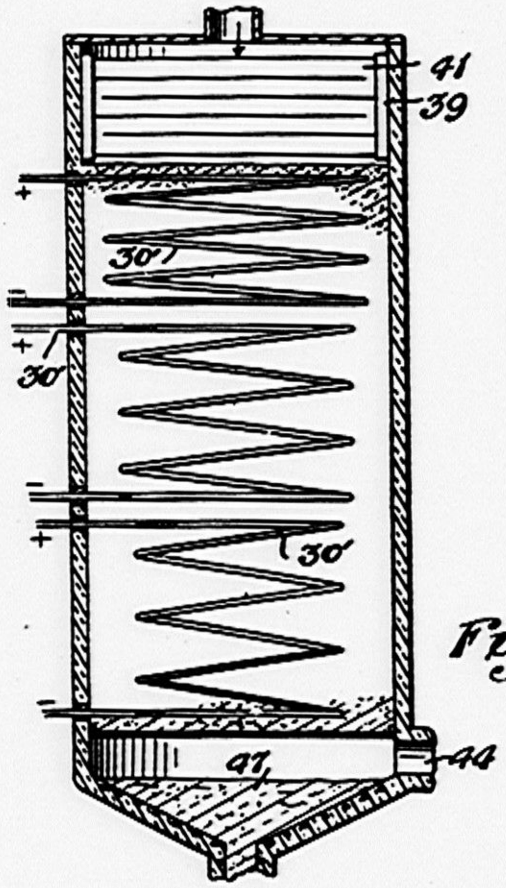


Fig. 8

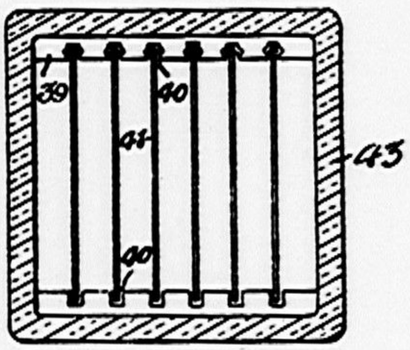


Fig. 7

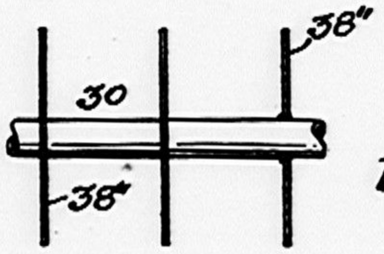


Fig. 9

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ADSORBENT APPARATUS

Original Filed Nov. 22, 1932 4 Sheets-Sheet 3

Fig. 4

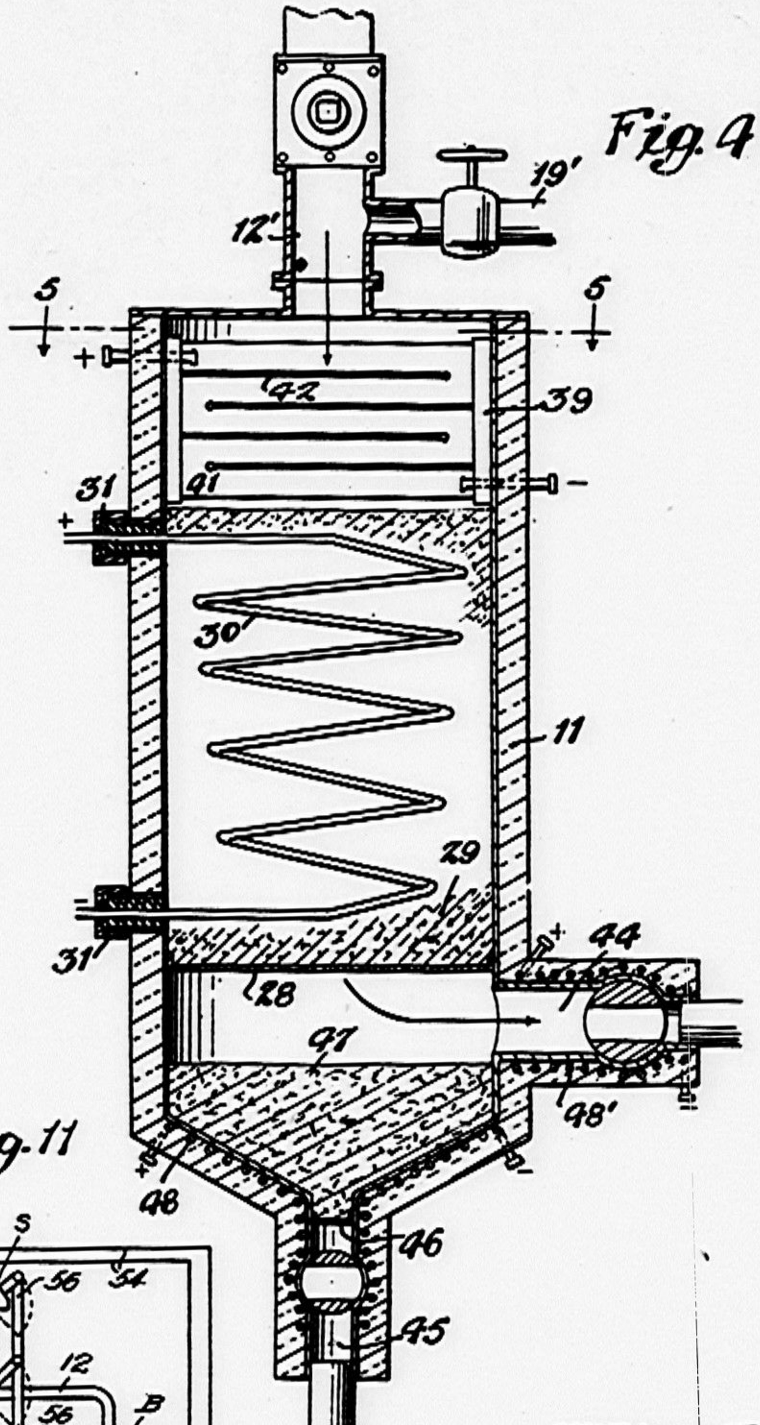
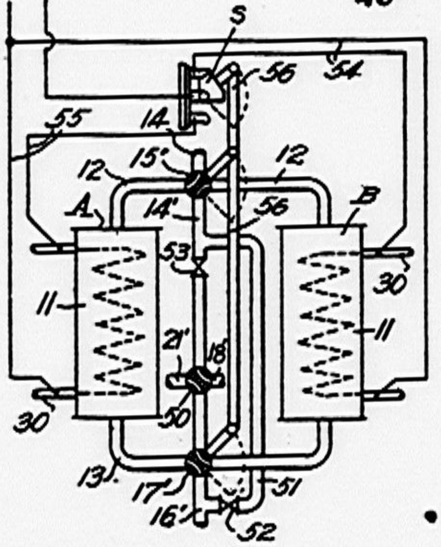


Fig. 11



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**June 15, 1937.** W. E. MOORE ET AL **2,083,732**

ADSORBENT APPARATUS

Original Filed Nov. 22, 1932 4 Sheets-Sheet 2

Fig. 2

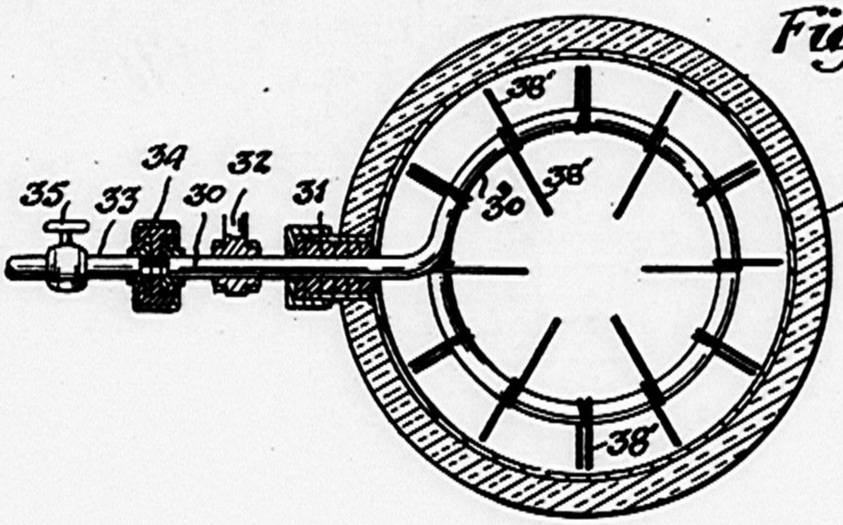


Fig. 5

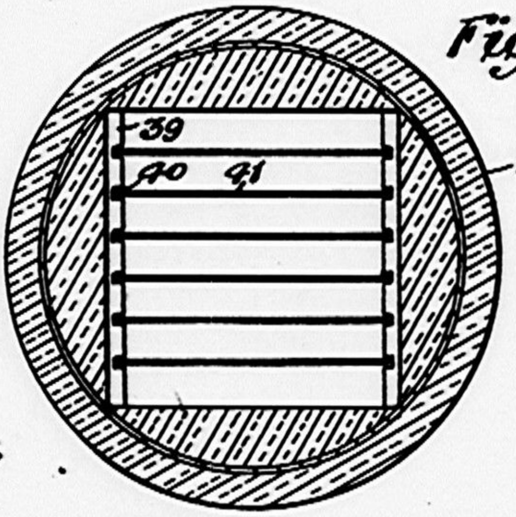
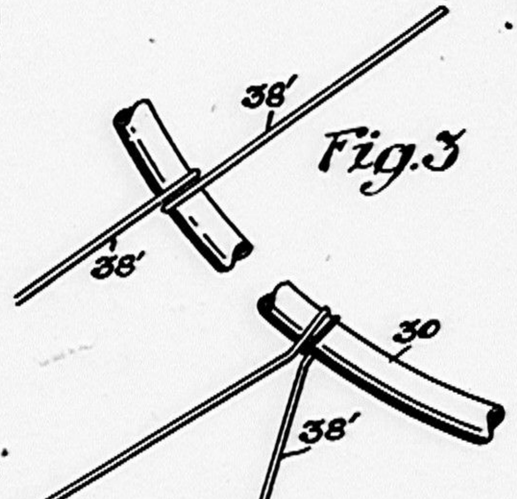


Fig. 3



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June 15, 1937. W. E. MOORE ET AL 2,083,732

ADSORBENT APPARATUS

Original Filed Nov. 22, 1932 4 Sheets-Sheet 1

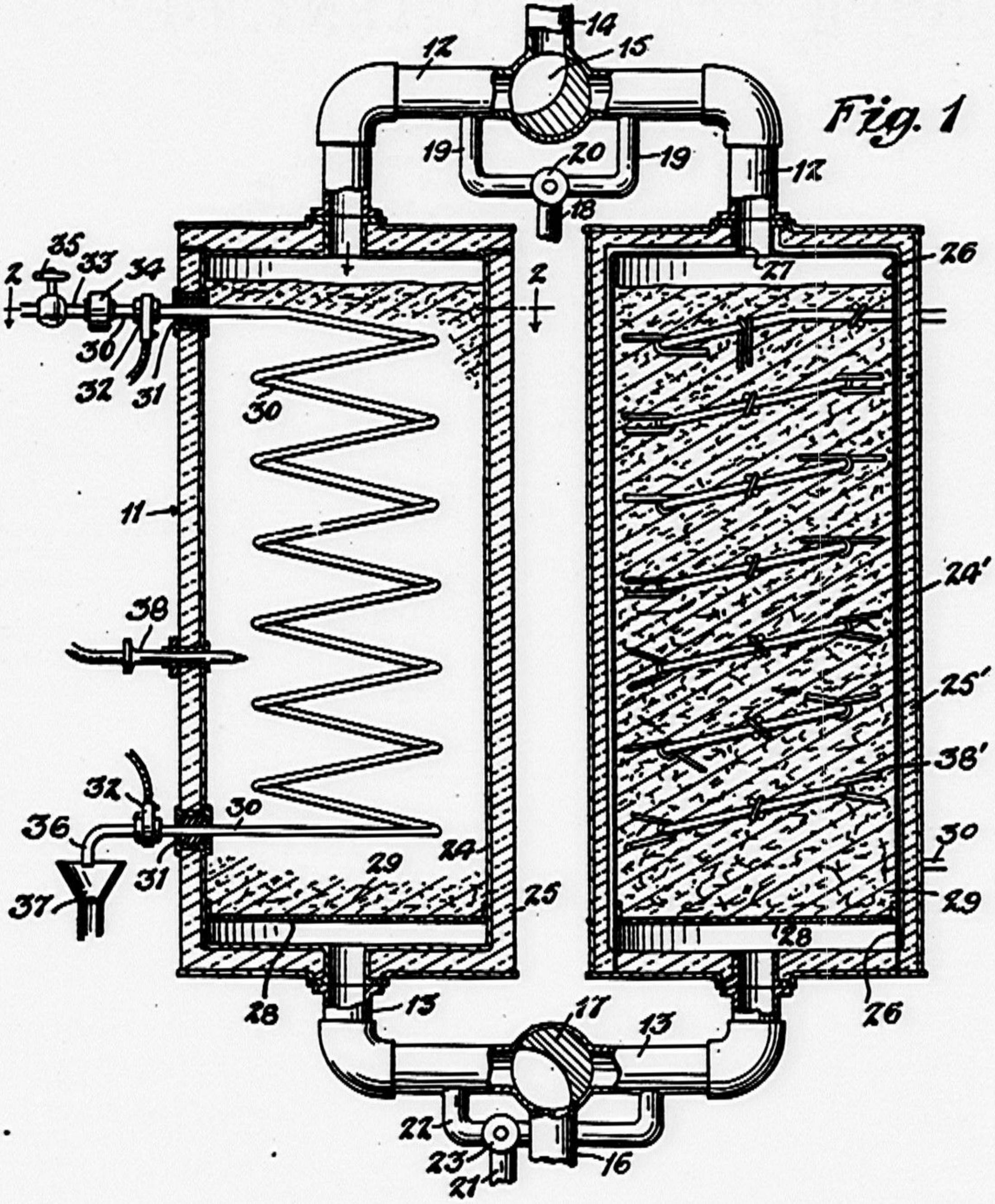

Fig. 1

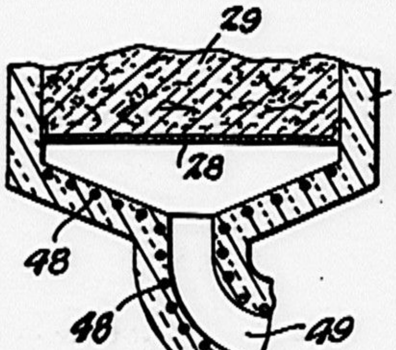

Fig. 10

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Patented June 15, 1937 **2,083,732**

# UNITED STATES PATENT OFFICE

2,083,732

## ADSORBENT APPARATUS

William E. Moore, Pittsburgh, and George L. Simpson, Coraopolis, Pa., assignors to Pittsburgh Research Corporation, Pittsburgh, Pa.

Original application November 22, 1932, Serial No. 643,940. Divided and this application March 3, 1936, Serial No. 66,948

21 Claims. (Cl. 183—4)

Our invention relates to improvements in apparatus for using adsorbents and more particularly to an apparatus for using solid adsorbent material.

To remove moisture or gas from air and gases for various technical and industrial uses, solid adsorbents such as activated alumina, adsorbent gels and the like are used. When the adsorbent material has extracted a certain percentage of their total weight of moisture or gas, it can remove no more moisture or gas until it has been reactivated. The reactivation process consists in driving off the moisture or gas which has been adsorbed.

Heretofore this reactivation has been accomplished by passing heated air through the adsorbent material; by heating the adsorbent with the hot gases from gas burners or the like, or by surrounding the container with a chamber containing high temperature steam. Experience has shown these methods of reactivation to be unsatisfactory.

An important object of our invention is to provide a novel apparatus for removing moisture or gas from gases or the like and to reactivate the adsorbent material after the adsorbing step.

Other objects and advantages of our invention will be apparent during the course of the following description.

This application is a division of our co-pending application 643,940 filed November 22, 1932 for Apparatus for using adsorbents.

In the accompanying drawings which form a part of this specification and wherein like characters of reference denote like parts throughout,

Figure 1 is a vertical sectional view through an adsorbent apparatus embodying our invention,

Figure 2 is a horizontal sectional view on line 2—2 thereof,

Figure 3 is a detail view of a portion of the heater element showing the position of the radiating spines thereon,

Figure 4 is a central vertical sectional view of a modified form of our invention,

Figure 5 is a horizontal section taken on line 5—5 thereof,

Figure 6 is a longitudinal sectional view of an air or gas heater for use with our adsorbent apparatus.

Figure 7 is a transverse section on line 7—7 of Figure 6,

Figure 8 is a sectional view of a further modification of our adsorbent apparatus,

Figure 9 is a detail of the heater element having a modified form of radiating member,

Figure 10 is a detail sectional view of a modification of the discharge end of our adsorbent apparatus, and,

Figure 11 is a diagrammatic view illustrating the valve arrangement for controlling the gas circulation, and the interconnection between the main control valves and the electric switch for controlling the heating means.

In the drawings, wherein for the purpose of illustration is shown a preferred embodiment of our invention, the numeral 11 designates the towers of a dual adsorbent unit connected at their tops by gas inlet pipes 12, and at their lower ends by gas outlet pipes 13. A supply pipe 14 is connected to the inlet pipes 12 by means of a three way valve 15 while a discharge pipe 16 is connected to the outlet pipes 13 by means of a three way valve 17. By means of valves 15 and 17 the pipes 14 and 16 may be connected to the left hand tower, as in Figure 1 or to the right hand tower, or completely shut off from both towers.

A wash gas supply pipe 18 is connected to each of pipes 12 by branches 19 which straddle valve 15 and is provided with a three way valve 20 to connect pipe 18 with either of the towers 11. A wash gas discharge pipe 21 is connected to pipes 13 by means of branches 22 which straddle valve 17, and a three way valve 23 is arranged to control the flow of wash gas from the towers.

The towers 11 may be of any suitable construction. At the left of Figure 1, we have shown a tower formed from a metallic casing 24 surrounded by refractory heat insulating material 25. The top of the tower may be removable if desired. This arrangement permits contact of the metal casing with the heated adsorbent material and therefore increases the length of time and amount of power required to reactivate the adsorbent material. It is preferred therefore to use the construction shown at the right of Figure 1, in which the metal casing 24′ is lined with heat insulating material 25′ A very thin walled metal container 26 is arranged inside of the heat insulating material. In this construction it is only necessary to heat these very thin metal walls to the temperature of reactivation, and not the heavy outer casing. Where the apparatus is operated at high pressures, a bleeder port 27 allows the pressure to equalize on both sides of the container. The outer casing supplies the mechanical strength, and the thin inner walls are subjected only to balanced pressures. The space between the inner metal walls 26 and the insulating lining 25' is exaggerated in Figure 1 to illustrate this balancing of pressures on both sides of the container 26.

While two forms of towers are illustrated in Figure 1, it is obvious that only one construction will be used in practice in a single unit, preferably the form shown at the right of Figure 1.

A perforated plate 28 extends completely across the lower end of each tower at a point spaced a short distance from the bottom of the tower and serves as a support for the adsorbent material 29 which may be activated alumina or other suitable material. The adsorbent material extends from the plate 28 to a point spaced a short distance below the top of the tower.

The heating element preferably comprises a metallic resistance element arranged in zig-zag formation, loops or spirals, and embedded in the adsorbent material. For convenience of illustration, the heating element is shown as arranged in spirals only. The resistance element is preferably tubular and extends from a point adjacent the top of the tower to a point adjacent the perforated plate 28. The ends of the heater element 30 extend through suitable insulating and packing glands 31 in the walls of the tower and project a substantial distance from the tower. Terminals 32 are clamped onto the heating element exteriorly of the tower and pass an electric current through the coil.

The upper end of the tubular heating element extends past the upper terminal and is connected to a fluid supply pipe 33 by means of a suitable insulating joint 34. A valve 35 controls flow of fluid from pipe 33 to the coil 30. The lower end of element 30 is turned downwardly at 36 and is arranged over the flared end of a discharge pipe 37. It will be seen that fluid may be passed through the coil 30 by operating valve 35, and will be discharged into pipe 37.

A suitable thermocouple 38 may extend through the walls of the tower to a point adjacent the heating coil 30 as seen in Figure 1.

In operation, the gas to be treated is passed downwardly through one of the towers until the adsorbent material has been saturated with moisture or whatever other element it is to remove from the gas stream. When this point of saturation is approached, three way valves 15 and 17 are operated to connect gas lines 14 and 16 to the other tower, and at the same time valves 20 and 23 are operated to direct wash gas through the saturated tower. In practice valves 15, 17, 20 and 23 may be interconnected to operate simultaneously. Electric power, the operating switch of which may also be interconnected to the valves to prevent inadvertent operation of the power, is then applied to terminals 32 and a current sent through the resistance element. Thermocouple 38 may then be used in the usual manner to automatically control the heating step and prevent overheating of the adsorbent material.

The adsorbed vapors are driven out of the adsorbent by the heating step and the stream of wash gas passing through the adsorbent continually disturbs the equilibrium in the adsorbent and carries away the vapors. When reactivation of the adsorbent is completed, the electric power is turned off and the wash gas is shut off. The tower may then be cooled either by natural heat loss, which is relatively slow, or by means of a cooling medium or refrigerant passed through the spiral heating pipe. The heating pipe may be made from "stainless" steel or other suitable material, to enable it to be used both for heating and cooling.

The heating element may be made sufficiently strong so as to be substantially self supporting, and thus dispense with complicated supporting means. When operated at low voltage, there will be no danger of short circuiting between adjacent turns of the coil, nor of other leakage.

To increase the rate of heat flow from the heating element to the adsorbent material, and to distribute the heat thoroughly through the adsorbent mass, radiating spines 38' of a high heat conductive metal are fastened to the spiral heating element as shown in Figures 2 and 3, and extend away from the heating element into the mass of adsorbent material. The spines are welded or brazed to the heating spiral to insure good thermal transfer, and are arranged at spaced intervals along the heating spiral, completely surrounding heating pipe 30, as seen in Figures 2 and 3. By this means heat generated in the heating element is conducted away from it into the mass of material. The heating element constructed thus takes up a minimum amount of the cross sectional area of the tower and so puts very little impedence in the way of the gas flowing through the towers. In Figure 11 is illustrated diagrammatically one method of interconnecting the main control valves and electric control switch S for simultaneous operation. The dual towers 11 of the system shown in Figure 1 are designated A and B for differentiation and are connected at top and bottom by pipes 12 and 13 as in Figure 1. In place of three way valves 15, 16, 20 and 23 we have provided a pair of four way valves 15' and 17' arranged in pipes 12 and 13 respectively. Supply pipe 14 is extended below valve 15' as pipe 14' and connects valve 15' with the valve 17'. A third four way valve 50 is arranged in pipe 14' and communicates with supply pipe 18' and discharge pipe 21' for the wash or reactivating gas. A by-pass pipe 51 connects pipe 14' above valve 50 with the gas discharge pipe 16' below valve 17'. A valve 52 of any suitable construction is arranged in by-pass 51 and a similar valve 53 is arranged in pipe 14' between four way valve 50 and by-pass 51.

It will be seen that when the four way control valves 15', 17' and 50 are in the position shown, and valve 53 is open while valve 52 is closed, wash or reactivating gas will enter four way valve 50 through pipe 18'. This gas will pass up through pipe 14' and thence through valve 15' and pipe 12 to adsorber B, down through adsorber B to pipe 13 and valve 17' and thence back to valve 50 and out through discharge pipe 21'. When it is desired to send the wash gas upwardly through the adsorber, valve 50 is rotated 90° so that gas entering through pipe 18' passes down pipe 14' to valve 17' and pipe 13 and passes upwardly through the adsorber. It is then returned through pipe 12, valve 15', pipe 14' and valve 50 to discharge pipe 21'. It will be seen therefore that valve 50 can be operated to reverse the direction of the gas through the adsorbers. The action of valve 50 is the same when valves 15' and 17' are operated to direct the gas from pipe 18' through adsorber A.

The switch S is connected by the circuit indicated at 54 to the heating coil 30 of adsorber B and by the circuit indicated at 55 to the coil of adsorber A. In the position of the switch shown in Figure 11 current is passing through coil 30 of adsorber B while the circuit to the coil of adsorber A is open. In order to prevent inadvertent movement of the switch while the adsorbers are in use, the control levers of switch S and valves 15' and 17' are interconnected by an operating link 56 so that valves 15' and 17' must necessarily act together. Furthermore, no special supports are required to hold the heating element in place and complicated insulators are therefore dispensed with. It will be seen that the spines also increase the efficiency of the cooling step as they will conduct heat to the cooling pipe.

The spines are not shown in the left hand tower of Figure 1 because the construction of the heating spiral is more readily apparent with the spines removed. These spines add greatly to the simplicity and efficiency of this apparatus both for heating and cooling. They are especially desirable because of the high heat insulating properties of the activated alumina or other adsorbent which hampers free transfer of heat.

In place of spines, fins or disks 38'' such as seen in Figure 9 may be used, and these disks may be integral with the pipe or attached thereto.

While we prefer to employ electric heating of the adsorbent, it is obvious that we may substitute other heating agents. For example, heating fluid such as steam or a hot liquid, may be circulated through the heating coil to produce this result.

A single unit may be used in place of the dual unit shown in Figure 1, especially where the apparatus may be run until some necessary pause in the process, such as night time or over the week-end shutdown, because of the rapid reactivation provided by our process.

When wash gas is admitted to the tower it is cool and therefore tends to cool the adsorbent at the top of the tower which consequently will not be as completely reactivated as will the adsorbent material near the lower end of the tower. This cooling effect of the wash gas may be compensated for in several ways. In Figures 4 and 5 we have illustrated electrical heating means for preheating the gas before it reaches the adsorbent material. A pair of resistance supports 39 of suitable insulating material are secured to opposite inner walls of the tower adjacent the top thereof, and are provided with vertically extending grooves 40 extending from the top of the supports 39 to a point adjacent but spaced from the bottoms of the supports. The grooves 40 are arranged at spaced intervals in the supports and slidably receive a plurality of plate resistors 41 which rest upon the bottoms of the grooves. The resistance plates 41 are spaced apart as seen in Figure 5 and are preferably provided with slits 42 alternately communicating with opposite sides of the plates, and the plates and supports are of the type disclosed in the co-pending application of George L. Simpson, Serial No. 585,994, filed January 11, 1932. Gas entering the tower through pipes 13' and 12' must pass by the heated resistor plates, and is split into thin films and passed through the heater in intimate contact with the heated surfaces of the plate resistors. To transfer energy from a heated surface to a gas stream, the washing action of the gas as it passes over the heated surface is an important factor, and this condition is utilized to the maximum with our construction. The heating elements are placed closely together so that a thin film of gas is exposed to the heating element on both of its sides. The velocity of the gas through the relatively narrow space between the surfaces of the heating elements scours off the gas in actual contact with the heated surfaces and insures efficient and rapid transfer of heat energy from the plates to the gas streams.

The arrangement of the spaced resistor plates provides a greater surface of heating element per cubic foot of gas than do the usual heaters and consequently permits rapid transfer of large quantities of heat energy at comparatively low resistor temperatures.

It is not necessary that the gas heater be arranged within the tower. In Figures 6 and 7 we have shown the heater arranged in the gas conduit 43 leading to the adsorbent tower or to any other apparatus desired. It is obvious that our heater may be used anywhere that a heated stream of air or other gas is desired. The supports are arranged in an enlarged portion of the conduit as in the tower shown in Figure 4 with the advantage that the plates 41 may be supported from their tops as in the Simpson application referred to above.

Another method of compensating for the cooling effect of the incoming air or gas is also shown in Figure 4. The turns of the heating coil 30 may be larger and/or closer together at the top of the tower than at its lower end and therefore will produce more heat near the top of the tower where the wash gas enters. In the drawings the coils are shown as both larger and closer together adjacent the top of the tower In Figure 4 this arrangement of the heating coils is shown as used in conjunction with the gas heating plates 41. It is obvious, however, that this arrangement of the heating coils may also be used independently of the gas heater and as a substitute therefor. The spines 38' or disks 38'' are also capable of use with this form of heating element.

When the heated moisture-laden wash gases reach the cooler parts of the tower, such as the bottom of the tower, and the valves and fittings, they will precipitate a considerable amount of the moisture they carry. If the flow of heated gases is continued long enough and fast enough to heat up these parts, all of the moisture will be removed. Such a result requires considerable time and gas, however. It is very desirable to prevent this precipitation of moisture, and the taking up of this moisture by the dried gases. In Figure 4 we have shown two methods of preventing this moisture precipitation, which may be used together, as in Figure 4, or independently. In place of using a common outlet for the dried gases, and the wash gases, a dried gas outlet 44 is provided in the tower wall at a point spaced from the bottom of the tower. The wash gas outlet 45 is arranged at the bottom of the tower, and in this form of the invention the bottom of the tower converges to the centrally arranged wash gas outlet 45. A perforated plate 46 is arranged adjacent or in the mouth of the outlet 45 and supports a layer or mass of adsorbent material 47 arranged between the perforated plate 46 and the dried gas outlet 44. Wash gas passes through the adsorbent mass 47 into the outlet 45 and any precipitation of moisture will occur in the mass 47 or on the fittings below dried gas outlet 44. Dried gas will pass between the upper perforated plate 28 and the mass 47 and out of outlet 44 without passing through the mass 47 and will therefore not take up any moisture.

Another method of producing this result is to place electrical heating elements around the parts which would otherwise be sufficiently cool to cause precipitation of moisture. The electric resistance elements **48** are arranged about the metal parts at the lower end of the tower in proximity to or in contact with the metal. These heater elements extend to a point below the outlet valve, and serve to heat the metals parts and prevent precipitation of moisture.

Similar heating elements **48'** may also be arranged in the dried gas outlet **44**. While the heater elements **48** may be used in conjunction with the mass **47** of adsorbent material, and the independent gas outlets **44** and **45** it may also be used alone, as shown diagrammatically in Figure 10, in which the mass **47** is dispensed with, and a single outlet **49** is provided.

This method of applying heat locally to the cooler parts of the tower greatly shortens the time for reactivation. It will be understood that the layer or mass **47** of adsorbent material, and the separate gas outlets may be used independently of the heater elements **48**.

In place of the heater coil **30** shown in Figure 4 in which variations in the turns of the coil are used, we may provide a heating element consisting of several sections providing separate zone control, so that the heating elements adjacent to the wash gas can be carried at a higher temperature than the heating elements farther along in the tower. This form of heater element is illustrated more or less diagrammatically in Figure 8 in which the sections **30'** are arranged independently. The heating sections may be of the same size, or may vary in size, as shown. While this form of heater is illustrated as used in conjunction with the gas heater **41** it may obviously be used independently thereof, in a tower such as shown in Figure 1.

An important feature of our invention is that the gas to be dried is passed downwardly through the towers. This downward passage of gas takes advantage of the fact that the tendency of the heat of adsorption to rise will localize the heated zone. Due to the fact that as a solid adsorbent takes up moisture it rises in temperature, an upwardly moving stream of gas would move in the same direction as the rising heat of adsorption, and the adsorbent material would consequently be heated ahead of the zone where adsorption is taking place. Where the gas stream passes downwardly, however, the heat of adsorption rises upwardly away from the downwardly progressing zone of adsorption and localizes the heating.

The scavenging or wash gas is also passed downwardly, and as a consequence we obtain the advantage of gravity removal of condensation. By passing the wash gas through the tower in the same direction as that taken by the gas to be dried, the exit point of the dried gas will be the driest part of the tower, as it tends to be the hottest point of the wash gas in the reactivation step. It is highly desirable therefore, that the dried gases exit from the same end of the tower as do the wash gases.

This effect may also be obtained by reversing the flow of wash gas through the tower at intervals, but finishing so that the wash gas exits from the tower in the same direction as the dried gas exits.

Where maximum freedom from moisture is desired, a portion of the dried gas from one tower may be circulated through the other tower as a wash gas during the final stages of reactivation so as to leave the adsorbent near the exit with a minimum amount of contained moisture. Referring to Figure 11 it will be seen that by closing valve **53** and opening valve **52** dried gas from the bottom of adsorber A will pass through valve **17'** to by-pass **51**, through open valve **52** to pipe **14'** and then through valve **15'** and pipe **12** to adsorber B.

An important feature of our method is the rapid reactivation of the adsorbent material. Heat distribution by means of the embedded heating element with its spines, provision to prevent collection of moisture in the outlet piping, the thin metallic container **26** to reduce the bulk of material to be heated, and the method of forced cooling as well as the provision for separating the gas outlet from the drain outlet by a layer of dried adsorbent are all features that operate to speed up the reactivation time.

When the moisture-laden gas is passed through activated alumina, or similar adsorbents, there is an evolution of heat energy equivalent to the heat of evaporation. This will cause a material rise in temperature unless the heat of adsorption is dissipated. It has been customary in the prior art to use proportionately large quantities of the adsorbent or to avoid heat insulation so that this natural rise will not reach a point which will reduce the capacity of the adsorbent to take up moisture. Very often the amount of moisture in a gas is variable and for certain periods of time may be very low in concentration so that a relatively small amount of adsorbent would effectively remove all of the moisture. If the concentration of moisture in the gas should increase greatly, however, this small amount of adsorbent would show such a rise in temperature as to reduce or completely destroy the ability of the adsorbent to take up moisture. To overcome these difficulties our invention may be provided with forced cooling at a controlled rate. This may be readily accomplished by forcing water, air or other cooling medium through the pipe or coil **30**.

It is only necessary to use this forced cooling when temperature rises in the material show that it is adsorbing moisture at a rate which prevents natural dissipation of the heat energy released. This may be easily determined by thermocouples suitably arranged, as at **38** in Figure 1. A cooling medium may be circulated to prevent the temperature rising above a predetermined point. This forced cooling allows the use of a minimum amount of adsorbent which will efficently dry gases the moisture content of which vary over a wide range.

While our invention has been described in connection with the removal of moisture from gases, it may be used to remove a gas from a mixture of gases or vapors, and for removing moisture from liquids, as in the dehydration of benzol and the drying of transformer oil or the treatment of lubricating oils. In some cases the reactivating cycle may involve washing the solid adsorbent by a solvent which may be introduced through the wash gas inlet and allowed to trickle down through the heated tower. The removal of the last traces of the solvent is accomplished by maintaining the heat and admitting the scavenger gas.

In some instances, better results may be obtained by maintaining a vacuum in the towers during reactivation.

While we have shown and described the preferred embodiment of our invention it is to be understood that various changes in the size, shape and arrangement of parts may be resorted to without departing from the spirit of our invention or the scope of the subjoined claims.

Having thus described our invention, what we claim and desire to protect by Letters Patent is:

1. A heating apparatus comprising an electrical resistance element, said resistance element having a longitudinal bore, and means to pass a cooling medium through said bore.

2. A heating apparatus comprising an electrical resistance element, said resistance element having a longitudinal bore, means to pass an electric current through said resistance element, a cooling fluid conduit, and an insulating member connecting said conduit with the resistance element and adapted to pass a cooling fluid from the conduit to the bore of the resistance element.

3. In a heating apparatus, a quantity of material to be heated and cooled, an electric heating element embedded in said material, said heating element being hollow, means to pass an electric current through said heating element to heat the material, and means to pass a cooling medium through the heating element to cool the material.

4. In a heating apparatus, a quantity of material to be heated and cooled, a hollow electrical resistance element arranged in coils and surrounded by said material, means to pass an electric current through said resistance element to heat the material, and means to pass a cooling medium through the hollow resistance element to cool the material.

5. A heating apparatus comprising a hollow electrical resistance element, high heat conductive members carried by said resistance element and radiating therefrom, means to pass an electric current through the resistance element, and means to pass a cooling medium through the resistance element.

6. A heating apparatus comprising an electrical resistance element, said resistance element having a longitudinal bore, high heat conductive members carried by said resistance element and radiating therefrom, a cooling fluid conduit, means to pass an electric current through the resistance element, and an insulating member connecting the conduit with the resistance element and adapted to pass a cooling fluid from the conduit through the bore of the resistance element.

7. In a heating apparatus, a quantity of material to be heated and cooled, a hollow electrical resistance element embedded in said material, high heat conductive members carried by said resistance element and radiating from the resistance element into said material, means to pass an electric current through the resistance element to heat the material, and means to pass a cooling medium through the resistance element to cool the material.

8. In a heating apparatus, a quantity of material to be heated and cooled, an electrical resistance element surrounded by said material, said resistance element having a longitudinal bore, high heat conductive members carried by said resistance element and radiating from the resistance element into said material, a cooling fluid conduit, means to pass an electric current through the resistance element to heat the material, and an insulating member connecting the conduit with the resistance element and adapted to pass a cooling fluid from the conduit through the bore of the resistance element to cool the material.

9. An adsorbent apparatus comprising a tower, a solid adsorbent arranged in said tower, an electric heating element embedded in said adsorbent, said heating element being hollow, and means to pass a cooling medium through said heating element.

10. An adsorbent apparatus comprising a tower, a solid adsorbent material arranged in said tower, an electric resistance element arranged in coils and embedded in said adsorbent, said resistance element being hollow, and means to pass a cooling medium through the resistance element.

11. An adsorbent apparatus comprising a tower, a solid adsorbent material arranged in said tower, and an electric resistance element arranged in coils and embedded in said adsorbent, said resistance element being hollow, whereby it is self supporting.

12. An adsorbent apparatus comprising a tower, an adsorbent material arranged in said tower, means to admit fluid to said tower, and a heating element embedded in said adsorbent material and distributed therethrough, the largest portion of said heating element being arranged in the portion of the adsorbent adjacent the point where fluid enters the tower.

13. An adsorbent apparatus comprising a tower, an adsorbent material arranged in said tower, means to admit fluid to said tower, and a heating element embedded in said adsorbent material and distributed therethrough, said heating element being arranged to produce a greater amount of heat adjacent the point where the fluid enters the tower than in other portions of the tower.

14. An adsorbent apparatus comprising a tower having a gas inlet end and a gas outlet end, an adsorbent material arranged in said tower, means to pass a gas through said tower and adsorbent material from the gas inlet to the gas outlet, and an electric resistance element embedded in said adsorbent material, there being a greater portion of the resistance element adjacent the gas inlet end of the tower than in other portions of the tower.

15. An adsorbent apparatus comprising a tower having a gas inlet end and a gas outlet end, an adsorbent material arranged in said tower, an electric resistance element embedded in said adsorbent material, there being a greater portion of the resistance element adjacent the gas inlet end of the tower than in other portions of the tower, and means to maintain the temperature of said adsorbent material below a predetermined value.

16. An adsorbent apparatus comprising a tower having a gas inlet end and a gas outlet end, an adsorbent material arranged in said tower, an electric resistance element embedded in said adsorbent material, there being a greater portion of the resistance element adjacent the gas inlet end of the tower than in other portions of the tower, said resistance element being hollow, and means to pass a cooling medium through said resistance element.

17. An adsorbent apparatus comprising a tower, an adsorbent material arranged in said tower, a heating element embedded in said adsorbent material, high heat conductive members radiating from said heating element into said adsorbent material, said heating element comprising a hollow electrical resistance member, and

means to pass a cooling medium through said resistance member.

18. In an adsorbent apparatus, a tower, an adsorbent material, means to pass a fluid through said tower and adsorbent material and a heating element embedded in said adsorbent material, said heating element being divided into a plurality of independent sections whereby the temperature in different parts of said tower may be independently controlled and regulated, said heating sections comprising electrical resistance elements.

19. In an adsorbent apparatus, a tower having a fluid inlet and spaced fluid outlets, an adsorbent material arranged in said tower between the inlet and outlets, means to heat the adsorbent material, a layer of adsorbent material arranged in said tower between the fluid outlets, and means to heat one of said outlets.

20. In an adsorbent apparatus, a tower adapted to receive a mass of adsorbent material, said tower comprising a metallic casing lined with refractory material, and a relatively thin metallic container arranged within said lining and in contact with the adsorbent material, said container having a bleeder port in one of its walls whereby pressure within the container will be balanced by pressure outside of the container.

21. In an adsorbent apparatus, a tower having a fluid inlet and spaced fluid outlets, a mass of adsorbent material arranged in the tower between the inlet and outlets, a layer of adsorbent material arranged in said tower between the fluid outlets, means to pass a fluid to be dried from said inlet through the mass of adsorbent material to one of said fluid outlets, means to heat the mass of adsorbent material, and means to pass a wash fluid from said inlet through the heated mass of adsorbent material and through the layer of adsorbent material to the other fluid outlet.

WILLIAM E. MOORE.
GEORGE L. SIMPSON.

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## From DEFENDANT'S EXHIBIT 1.

**June 27, 1939.** E. W. FLOSDORF **2,163,996**

LABORATORY APPARATUS

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Fig. 8.

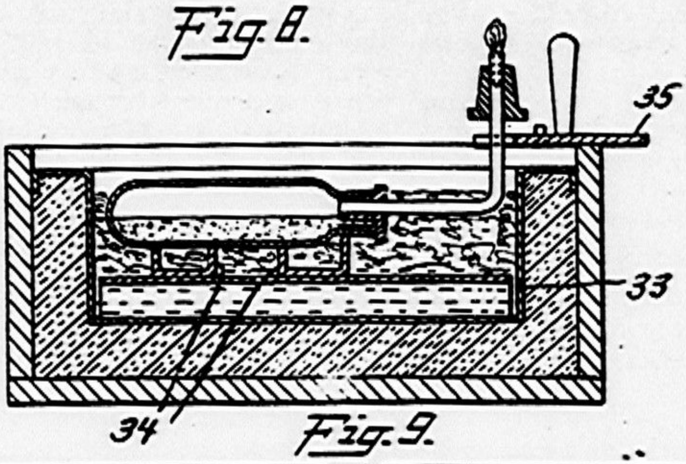

Fig. 9.

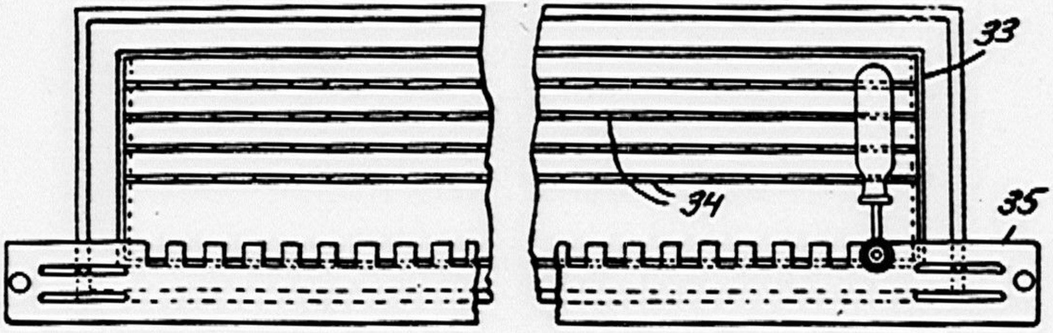

Fig. 10.

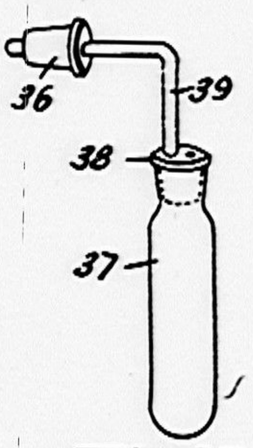

Fig. 11.

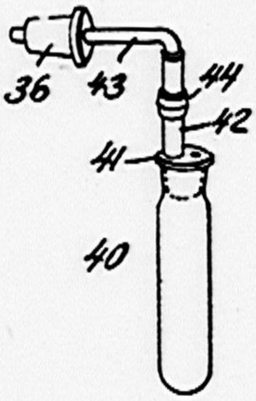

Fig. 12.

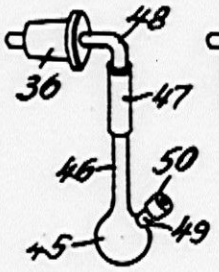

Fig. 13.

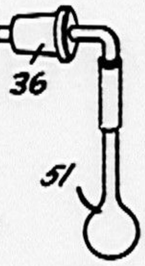

Fig. 14.

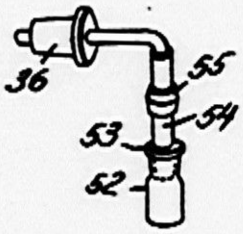

Fig. 18.

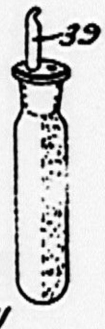

Fig. 19.

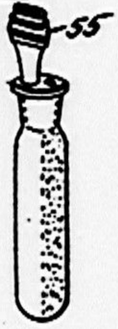

Fig. 15.

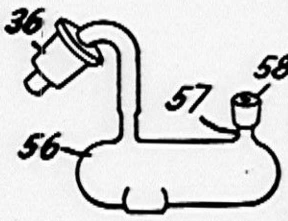

Fig. 16.

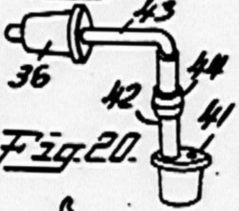

Fig. 17.

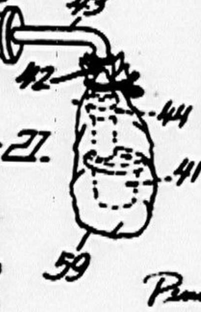

Fig. 20.

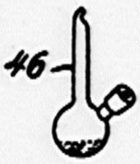

Fig. 21.

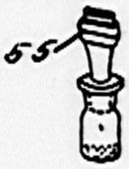

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Fig 5.

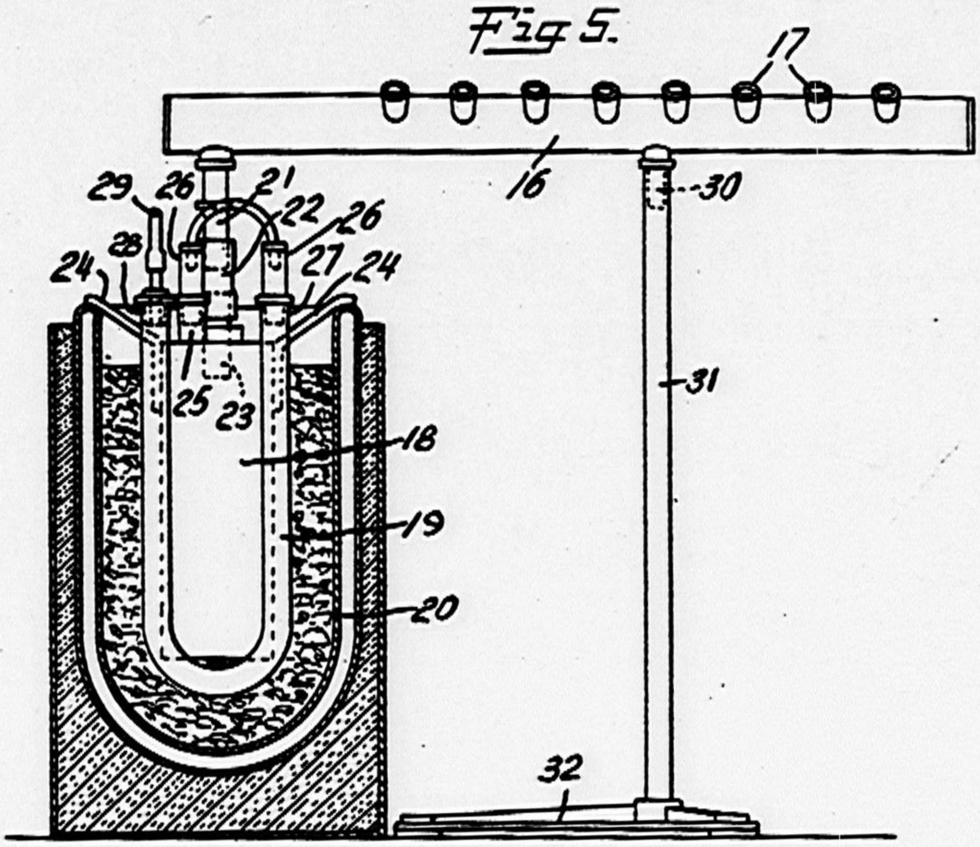


Fig. 6.

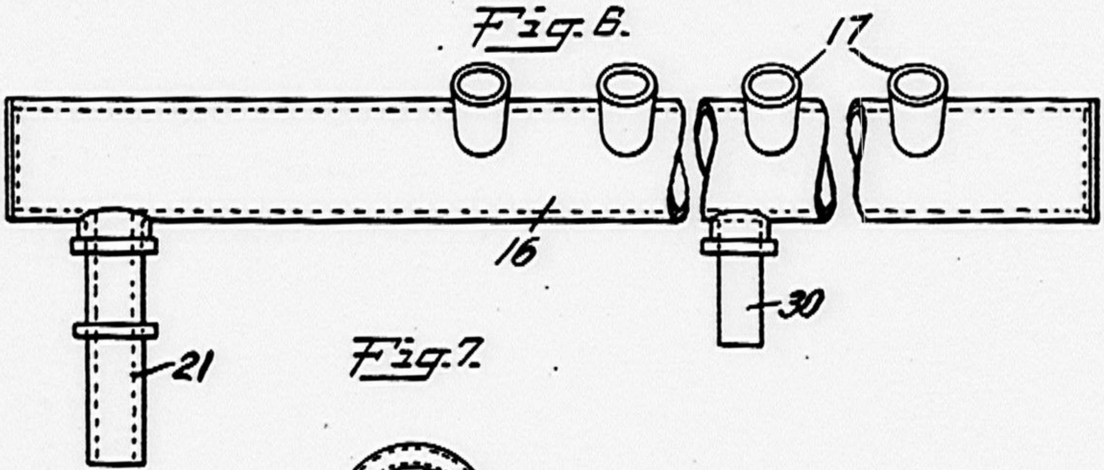
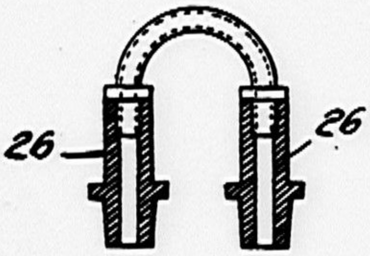


Fig. 7.



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**June 27, 1939.** E. W. FLOSDORF **2,163,996**

LABORATORY APPARATUS

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Fig. 2.

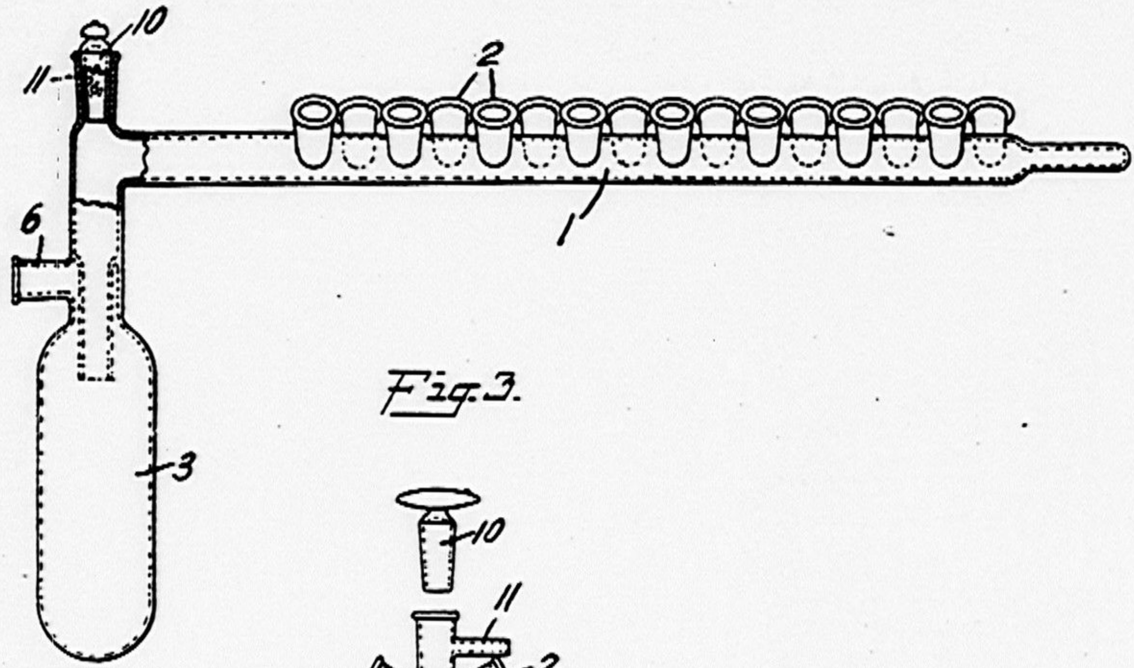


Fig. 3.

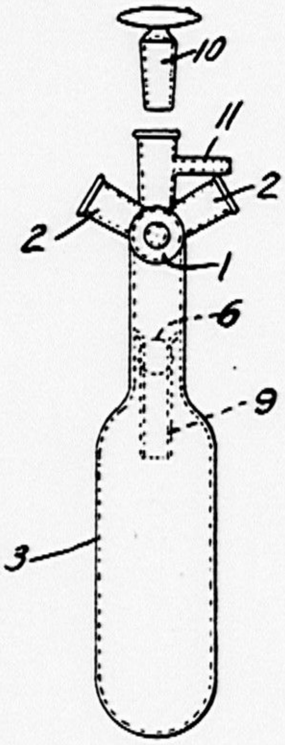
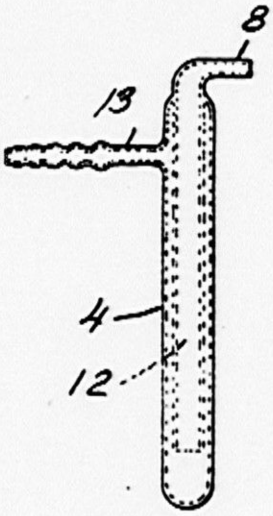


Fig. 1.



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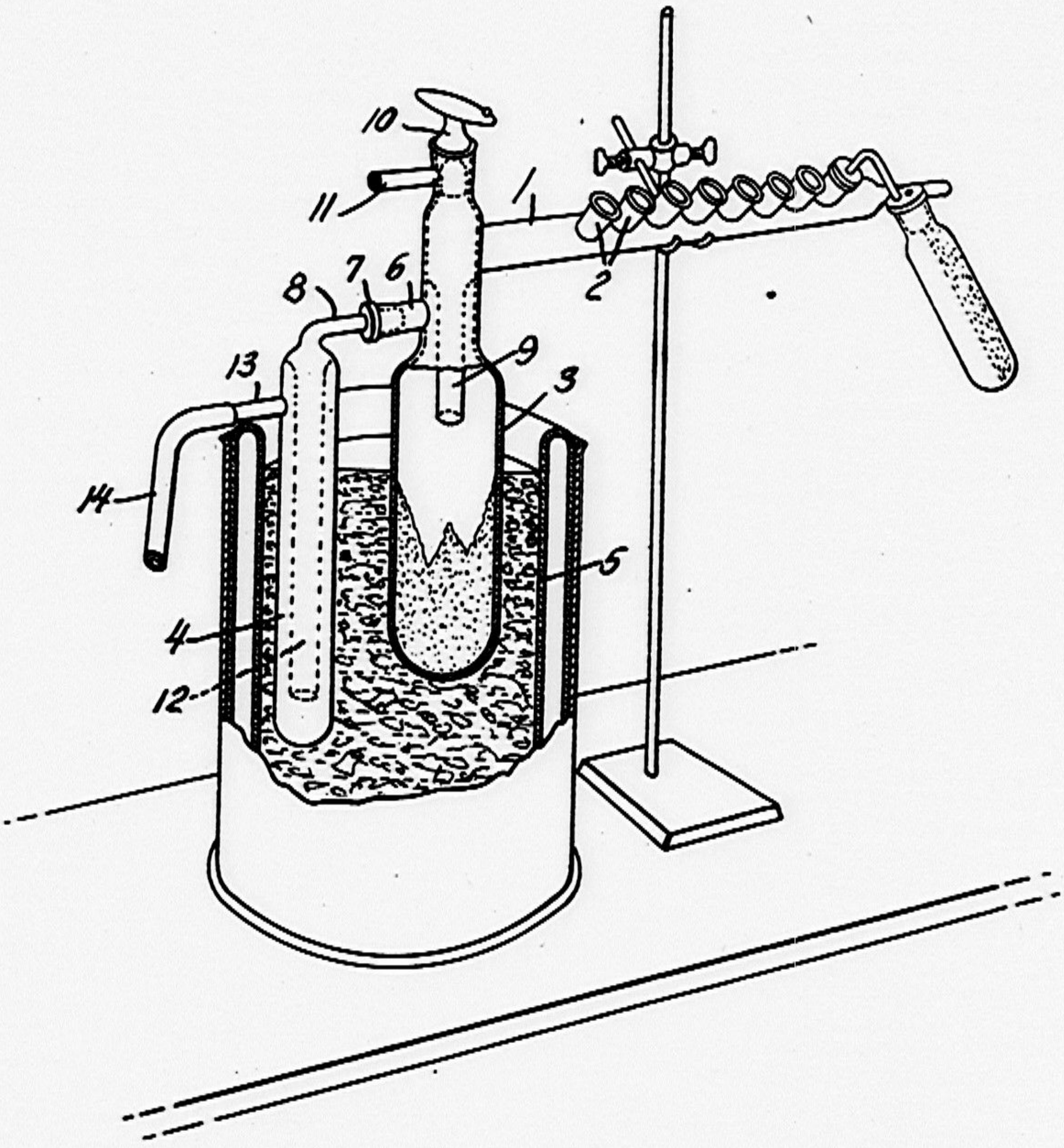
LABORATORY APPARATUS

Filed Dec. 14, 1935 4 Sheets-Sheet 1

Fig.1.



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**Patented June 27, 1939** **2,163,996**

# UNITED STATES PATENT OFFICE

2,163,996

## LABORATORY APPARATUS

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Application December 14, 1935, Serial No. 54,450

7 Claims. (Cl. 34—3)

This invention relates to improvements in apparatus for the preservation of biologically active substances such as sera, protein solutions, bacterial cultures, viruses and other labile biological substances; and more particularly to improvements in apparatus for the treatment and preservation of biologically active substances by freezing the substance, dehydrating it from the frozen state under a high vacuum, and carrying out the operation in the final individual containers in which the resulting product is to be kept until used.

In my companion applications, Serial Nos. 54,148, filed December 12, 1935, and 54,149, filed December 12, 1935, I have described such methods and apparatus for the preservation of such biologically active substances, and more particularly methods and apparatus for carrying out such preservation on a larger scale. The present invention relates more particularly to miniature laboratory apparatus for the carrying out of such processes and is intended primarily for research and experimental purposes where only a small amount of the biological materials are to be preserved.

The improved processes, set forth in detail in said companion applications, involve the treatment and preservation of the biologically active substances in the final container, with introduction of the substance in a liquid or semi-liquid state into the final container, freezing of the substance therein, dehydration of the substance from a frozen state and without melting, and with continuation of the dehydration until the final dry product is produced with the use of a high vacuum, and sealing the container at the end of the process. The process is particularly valuable for preserving small unit or multiple unit amounts of the biological substance in small individual containers in which the final product is to be preserved and kept until it is to be restored for use. The process thus makes possible the preservation of small and clinically useful amounts or units, or multiple units, of serum and other biologically active substances. The improved apparatus of the present invention enables such substances to be produced in the laboratory for research, experimental and similar purposes, on a small scale, and with similar advantages to those obtained in larger scale production according to the methods and apparatus of my said companion applications.

The invention is particularly valuable for the production of so-called "lyophile" products by the rapid and complete freezing of the liquid product at a low temperature, e. g. around —70° C. or lower, and with rapid vacuum evaporation from the frozen state and with self-regulation and automatic regulation of the temperature to keep the material frozen during the evaporation, even though exposed to room temperature.

The apparatus of the present invention comprises a manifold which is advantageously made integral with a main condenser, a secondary condenser attached to the main condenser by a rubber connection, a vacuum pump or other suitable vacuum producing means, small containers for the material to be preserved and suitable means for attaching the containers to the outlets of the manifold.

In one form of the apparatus the manifold and main condenser are made of glass in the form of an integral unit, and with a separate, secondary glass condenser attached to the main condenser by a suitable rubber connection.

In another form of the apparatus the manifold and condensers are made of metal, and the manifold and main condenser may likewise, and with advantage, be made as an integral, unitary structure.

The manifold of the apparatus, whether of glass or of metal, has a number of outlets of a shape and arrangement to receive a rubber stopper through which the exhaust tube of the individual containers extend, and with the outlets of the manifold and the rubber stoppers accurately proportioned to enable a vacuum-tight connection to be effectively and almost instantly obtained.

The main and auxiliary condensers are constructed and arranged so that they may both be contained within the same low temperature bath and the main condenser is so constructed as to insure effective condensation of most of the water vapor therein, so that only a small amount of the water vapor is permitted to go to the secondary condenser, and so that the secondary condenser can effectively remove the last of the water vapor and serve as a protection for the vacuum pump, making unnecessary the provision of an absorbent tube of dehydrating material such as $P_2O_5$ between the condenser and the pump.

The arrangement of the condensers in the container which contains the refrigerating mixture surrounding the condensers is such that automatic sublimation of ice from the upper part of the condenser to the lower part of the condenser is obtained during the carrying out of the process and as the level of the refrigerating bath is lowered, due to removal of refrigerant, e. g., solid carbon dioxide or Dry Ice.

The refrigerating mixture is advantageously one made up of Dry Ice or solid carbon dioxide suspended in a suitable organic liquid such as acetone, alcohol, ether, trichlorethylene, etc., but advantageously a bath containing the solid carbon dioxide and Methyl Cellosolve (the methyl ether of ethylene glycol). By surrounding the main and auxiliary condensers with such a bath, and by proper construction of the condensers, the water vapor removed from the frozen material, during the process, under the high vacuum maintained, is effectively condensed without objectionable stoppage of the tubes through condensation of ice at parts of the apparatus where such condensation is undesirable.

It is important, in a small laboratory apparatus, where the parts of the apparatus are small, to have a construction and arrangement of the parts such that the process can be carried out in a reliable manner and with a minimum of attention and also with a minimum of danger of stoppage of the apparatus by condensation of ice in connecting tubes or pipes, while insuring effective condensation in the main condenser.

The individual containers for the biological material to be treated and preserved may be small unit containers such as are described in my said companion applications, with suitable exhaust tubes, suitably proportioned, and with containers of proper shape and size, to enable the process to be effectively carried out; and the exhaust tubes of these containers are provided, in accordance with the present invention, with one-holed rubber stoppers, accurately proportioned with reference to the openings in the manifolds, so that the stoppers can be quickly thrust into the tapered outlets of the manifold and secured therein with a vacuum-tight fit.

The vacuum pump or other vacuum producing means used should be such as will enable a high vacuum to be maintained, e. g. below 0.70 mm. mercury or desirably much below that pressure, e. g., within the range of from 0.01 to 0.05 mm. mercury. This range is easily attained with a suitable vacuum pump provided precautions are taken to have high vacuum-tight connections throughout the apparatus. To attain this result in a small laboratory apparatus it is important to have rubber tube and rubber stopper connections, where used, properly proportioned and arranged to accomplish this result.

The individual containers for the biological material may be of various types and can readily be standardized for laboratory and research purposes. For the preservation of sera and other protein solutions, etc., containers of 50, 25, 10, 5 and 2 ml. capacity have been standardized; containers of larger capacity can be used, but containers of the capacity mentioned are sufficient for the usual laboratory and experimental operations. Containers of 2 and 5 ml. capacity have been found convenient for preservation of materials in small amounts, e. g., virus suspension in amounts as small as 0.1 ml. The volume of material put into any container should not exceed about a half the capacity of the container. For amounts of materials in excess of about 1 ml. the containers are advantageously in the form of cylindrical glass containers in which the material can be frozen while the container is in a horizontal position, so as to give a proper ratio of surfaces and volume of the frozen material, as more fully explained in my said companion applications.

The invention will be further described in connection with the accompanying drawings which illustrate, in a somewhat conventional and diagrammatic manner, laboratory apparatus embodying the invention; but it will be understood that the invention is not limited thereto.

In the accompanying drawings:

Fig. 1 is an elevation, with parts in section, and parts omitted, showing a glass apparatus, the vacuum pump being omitted, and one of the individual containers being shown connected to the manifold;

Fig. 2 shows an integral manifold and main condenser in elevation and with parts in section;

Fig. 3 is an end view of part of the apparatus of Fig. 2;

Fig. 4 is a separate view of the secondary condenser;

Fig. 5 is a view similar to Fig. 1 showing the apparatus made of metal;

Fig. 6 shows the separated manifold of Fig. 5 on somewhat larger scale;

Fig. 7 is an enlarged view showing the connection between the main and secondary condensers of Fig. 5;

Fig. 8 is a cross-section of a freezing trough with the freezing mixture and an individual container shown therein;

Fig. 9 is a plan of a freezing trough, also showing one individual container therein;

Figs. 10 to 15 show different forms of containers with rubber stoppers attached for connecting these containers with the outlets of the manifolds;

Fig. 16 shows one form of separated attachment, separated from the container;

Fig. 17 is a similar view showing a separated attachment of Fig. 16 as protected during sterilization and before attachment to the container; and

Figs. 18 to 21 show different forms of a sealed final container with the preserved material therein.

The apparatus of Figs. 1 to 4 is a glass apparatus to be made, e. g. of Pyrex glass. The manifold 1 has a number of tapered outlets 2, which may vary in number in laboratory apparatus of varying size, e. g., from 8 to 24 outlets. The manifold is shown as integral with the main condenser 3.

The main condenser 3 and secondary condenser 4 are enclosed within the same container 5, shown as a double-walled container with a vacuum between the walls for heat insulation purposes. The container 5 contains the freezing mixture, e. g., of Dry Ice and Methyl Cellosolve, surrounding the main and secondary condensers.

The connection between the main and secondary condenser is through a side outlet opening 6 having an accurately fitting rubber stopper 7 therein through which extends the inlet tube 8 of the secondary condenser.

The main condenser has an inlet tube extension 9 which extends down into the body of the condenser to a limited extent and terminates at the level of the top of the container 5 and the main condenser is supported in the container so that it extends to this level but does not extend further. The outlet tube 6 is arranged at a higher level. The arrangement of inlet and outlet tubes is such that the tendency to short circuiting of the vapors through the primary condenser to the secondary condenser is prevented or minimized, and also such that the inner tube extension 9 is protected from plugging by the deposit of ice therein. The main condenser has a tightly fitting stopcock 10 at its top with a side tube 11 through which, and through the stopcock when in proper position, air may be admitted to destroy the vacuum at the end of the process.

The secondary condenser has its inlet tube 12 extending to near the bottom and has its side outlet 13 near its upper end. With effective operation in the main condenser, the amount of water vapor carried over to the secondary condenser is so small that danger of plugging of the secondary condenser with ice is obviated. The rubber stopper connection between the main and secondary condensers, and the opening receiving this stopper, are accurately proportioned to insure a vacuum-tight fit. The secondary condenser is connected through the side outlet 13 to a heavy rubber tubing 14 leading to the vacuum pump (not shown).

In the apparatus of Figs. 5, 6 and 7 a manifold 16, with its tapered outlets 17 and the main and secondary condensers 18 and 19 are made of metal, these condensers being enclosed within a container 20, shown as a double walled vacuum container surrounded by insulation.

The manifold 16 has at one end an outlet connection 21 leading to the main condenser, with which it may be integrally united by brazing or otherwise or with which it may be connected through a suitable rubber tube connection shown at 22 which surrounds the lower end of the manifold outlet 21 and also the tubular extension 23 at the top of the main condenser, as shown. This tubular extension 23 which forms a part of the inlet to the main condenser extends down about ½" to 1" below the top of the condenser when this condenser is arranged as shown in Fig. 5. The condenser is suitably supported in the container 20 by supports 24 so that its top is about 1" below the top of the container 20. The main condenser has an outlet connection 25 connected through the rubber tube 26 to the inlet 27 of the secondary condenser.

The secondary condenser is of general U-shape and is arranged in the container 20 at one side of the main condenser. Its outlet 28 is adapted for the connection of a rubber tubing 29 leading to the vacuum pump (not shown).

The manifold of Fig. 5 is shown as having an integral extension 30 adapted to extend into a supporting pipe 31 carried by stand 32 for supporting the manifold.

The number of tapered outlets on the manifold of Figs. 5 to 7, as well as the number on the glass manifold of Figs. 1 to 4 can be somewhat varied to provide for the attachment of a larger or smaller number of individual containers.

A suitable freezing trough, for freezing the serum or other biological material in the individual containers is shown in Figs. 8 and 9, the trough 33 having individual supports 34 for the individual bottles or containers, and a rocking device 35 by means of which if desired the containers can be rocked back and forth by movement of the upwardly projecting portions of the L-shaped exhaust tubes.

Various shapes of containers suitable for use in the practice of the invention are shown in Figs. 10 to 15, each having a rubber stopper 36 on the exhaust tube of the container for attaching the container to an outlet of the manifold. The container 37 of Fig. 10 has a rubber stopper closure 38 and an L-shaped exhaust tube 39 which also extends through the rubber stopper 36.

The container 40 of Fig. 11 has a special rubber stopper 41 with an integral tubular extension 42 which is attached to one end of an L-shaped exhaust tube 43 which in turn extends through the rubber stopper 36.

A clamping device of metal is shown at 44 of Fig. 11 surrounding the tubular extension 42 and adapted, when the process is completed, for sealing this tube by compression of this metal clamping device against the tube.

The container 45 of Fig. 12 is spherical in shape with a tubular extension 46 shown as connected through the tube 47 with the L-shaped glass exhaust tube 48. The container 45 also has a side extension 49 with a perforable rubber stopper 50 closing the same with a vacuum-tight seal.

The container 51 of Fig. 13 is similar to that of Fig. 12, and the other parts are similar, except for the omission of the side extension 49 and stopper 50.

In Fig. 14 the small container 52, which is of smaller size than the container of Figs. 10 and 11, has a smaller rubber stopper 53 with integral tubular extension 54 having clamp 55 thereon similar to the clamp shown in Fig. 11.

The container 56 of Fig. 15 is of somewhat different shape and has the stopper 36 attached directly to an integral tubular extension of the container and has also another connection 57 with rubber stopper 58 therein.

Fig. 16 shows the rubber tube stopper, exhaust tube and manifold stopper of Fig. 11 separated from the container. These new rubber stoppers, and final container sealed by means of them, are claimed in my companion application Serial No. 106,105, filed October 17, 1936. Fig. 17 shows the same or a similar assembly of container stopper, exhaust tube and manifold stopper with a protective bag 59 of paper or cloth surrounding the stopper. This is used for sterilizing the assembly before use and protects the sterilized stopper until it is to be inserted in the container which is separately sterilized before the liquid material is placed therein. By sterilizing the container and the stopper and by moistening the stopper with sterile distilled water or dilute antiseptic solution when it is inserted, the material in the container is protected from contamination and asepsis is maintained.

The L-shape of the exhaust tube and the upwardly extending arrangement of the manifold outlets causes the exhaust tubes to have a downward direction when inserted in the manifold and this, together with the rapid flow of vapor from the containers through the exhaust tubes, and the protection of the containers from ingress of air at the end of the process, by sealing them under their original vacuum, maintains asepsis throughout the process and insures that the material originally placed in the container in a sterile condition will be protected from contamination, with certainty, up to the time it is restored for use. In Fig. 17 is also shown a cotton plug in the outlet and of the exhaust tube. This is left in the tube during the freezing and until the tube is to be attached to the manifold and the cotton plug is then removed, and by having the tube directed downwardly at this time and until it is placed in the manifold outlet and secured therein, there is no possibility of ingress of material through the exhaust tube and into the container.

Figs. 18 to 21 show the containers of Figs. 10, 11, 12 and 14 after the containers have been sealed, at the end of the process, the sealing of the containers of Figs. 19 and 21 being by heavy compression of the clamps 44 and 55 and by cutting off the rubber tubes above the clamps; while the sealing of the containers of Figs. 18 and 20 is by fusing and drawing of the glass exhaust tube 39 of Fig. 10 and of the integral tubular extension 46 of Fig. 12.

In the operation of the apparatus the serum or other biological material in unit or multiple unit amounts is introduced into the small individual containers, the containers are then closed and the rubber stoppers or other connections added thereto to connect the containers with the exhaust tubes which are L-shaped and each of which is provided with a one-holed rubber stopper which may be quickly thrust into the tapered outlets of the manifold with a vacuum-tight connection. The material is then frozen and while in a frozen state is subjected to vacuum dehydration with automatic self-regulation of temperature and this operation is continued until the dehydration has been carried to the proper extent which is attained after the removal of ice is completed and the product is thereafter warmed up to around room temperature and kept at that temperature while the vacuum is maintained to insure completion of the dehydration.

The freezing of the material is advantageously carried out by rapid freezing to a low temperature such as by insertion of the small containers, with their liquid content of biological material in a bath of Dry Ice suspended in an organic liquid and by leaving the containers in this bath for a considerable time to insure thorough freezing and the establishment of equilibrium at a low temperature. This rapid freezing followed by proper rapid dehydration gives a valuable lyophile product.

The freezing is carried out, in the apparatus illustrated, by placing the cylindrical container on its side in the bath of Dry Ice and Methyl Cellosolve with the exhaust tube extending vertically, as illustrated in Fig. 8. Care should be exercised not to freeze the serum or other material over the end of the exhaust opening. After solidification of the serum the containers are well covered with Dry Ice lumps and allowed to stand for about half an hour thus bringing them to a low temperature such that thawing will not occur during the attachment of the containers to the manifolds and during subsequent evacuation down to the working range at which evaporation automatically keeps the material frozen, e. g., at a vacuum below about 0.7 mm. mercury. Not even the surface of the serum should be allowed to melt or otherwise frothing will occur. For processing the material in an effective and reliable manner, the size and shape of the cylindrical containers are advantageously such that the layer of frozen material on the side while in a horizontal position therein is not less than 3 millimeters in thickness at the vertical diameter nor more than 15 millimeters, and also such, as already pointed out, that the volume of the frozen material does not exceed about one-half the volume of the containers. When amounts on the order of 25 ml. in a 50 ml. container are frozen, the container should be rocked somewhat during freezing so that a trough will form in the center, thereby reducing the thickness and increasing the evaporation area.

In the operation of the apparatus, Dry Ice or solid carbon dioxide broken into lumps approximately ½" in diameter is placed in the container around the condensers up to the mouth of the container. The organic liquid, e. g., Methyl Cellosolve, is then poured in slowly to within a short distance of the top of the container in Fig. 1, e. g., about 2" below the top in the apparatus shown where the main condenser has an outside diameter of about 65 mm. In the apparatus of Fig. 5 the organic liquid is filled into the container to within about ¼" or ½" of the flat top of the main condenser. The Dry Ice consumed in chilling the condensers at the outset of the process is replenished but no further additions are made until near the end of the process, e. g., for about eighteen hours.

After each container is removed from the freezing bath, the cotton plug, shown in Fig. 8, is removed from the end of the exhaust tube, the special manifold stopper on the top is lubricated with sterile or distilled water or dilute antiseptic solution and the stopper is then quickly thrust with a twisting motion deep into one of the outlets on the manifold. This requires only two or three seconds per container. Rapid attachment is essential in order to avoid surface thawing especially when the containers have amounts less than about 5 ml. in them. When all the manifold outlets are thus filled the vacuum pump is started immediately. If some of the outlets are not connected with containers, they should be plugged with a solid rubber stopper with a vacuum-tight fit. Within a short time the outsides of the containers usually begin to frost, due to condensation and freezing of atmospheric moisture. From this point on the process proceeds automatically with no need for further attention until the Dry Ice is replenished after about eighteen hours. As dehydration nears completion and the evaporation rate diminishes, the temperature of the containers slowly rises to that of the room and the frost thaws. The process is continued for some time thereafter to insure completion of the dehydration while the material is at a high temperature, and with a high differential between the vapor tension of the water in the material at the high temperature and the low vapor tension in the condenser.

In freezing the material in containers having small amounts, less than 1.0 ml. per container, as in the case of bacterial vaccine, for example, freezing of the container on its side is unnecessary in order to secure a large evaporating surface and with such small containers it is advantageous to have the freezing pans in such a position that the material can be frozen after attachment of the containers to the manifold but prior to evacuation. With small single containers containing such a small amount of material, it is of advantage to keep the containers in the freezing mixture until the proper degree of evacuation has been obtained and the automatic temperature control is secured. The process is thereafter carried to completion with automatic temperature control, as hereinbefore described. With larger containers containing a somewhat larger amount of serum or other material, and with the ratio of volume and surface hereinbefore described, and in containers of proper size and shape, the containers can be rapidly attached, after removal from the freezing bath, to the manifold, and the manifold rapidly evacuated to start self-refrigeration or automatic temperature control.

At the outset of the process most of the frozen condensate collects near the top of the main condenser but as the bath level around the condenser falls, due to consumption of the Dry Ice in condensing the vapor, the condensate sublimes downwardly in the condenser. In this way the entire bottom portion of the condenser is filled. Replenishing of the Dry Ice is undesirable during this stage of the process as it would interfere with or prevent this resublimation in the condenser. As the level of the solid-liquid mixture in the cold bath around the condensers becomes lower, the cold and heavy carbon dioxide gaseous atmosphere above the mixture will still extend to the top of the container and consequently to the level of the bottom of the inlet tube in the main condenser. This carbon dioxide atmosphere is of sufficiently low temperature to cause preliminary freezing in the main condenser of the vapor coming over and to prevent short circuiting of the vapor over into the secondary condenser. The condensate consequently assumes a shape and position such as illustrated in Fig. 1. Ordinarily the condensation in the main condenser is sufficiently complete so that only about 1% of the total condensate collects in the secondary condenser. In order to facilitate the removal of the last traces of moisture from the substances being dehydrated it is advisable to replenish the Dry Ice in the container around the condenser near the end of the process, e. g., after about eighteen hours, somewhat more or less, depending somewhat upon the size of the apparatus and the operation of the process.

By following the procedure described, as much as 99.96% of the original water content of serum may be removed, giving a product containing only about 0.5% of moisture in the final product as determined by desiccation in an oven at 110° C. The time required to obtain this degree of desiccation depends on the quantity of serum or other material to be dried. Amounts of the order of 0.1 ml. per container are dry within a few hours; while amounts of serum, etc., up to 25 ml. can be dried in around eighteen hours, although it is safer to allow a longer period, e. g., of about twenty-two hours. This enables the apparatus to be kept operating on a daily schedule of twenty-two hours process time, allowing two hours for sealing off one set of containers and preparing the containers for the next operation.

The last step of the process is that of sealing off the containers without breaking the vacuum. If glass exhaust tubes are used the seal is made with a suitable oxygen torch fitted with a double tip. By careful, slow and even heating of the tube on all sides at once, while pulling on the glass, an excellent seal of Pyrex exhaust tubes up to 5 mm. internal diameter is readily obtained, giving a sealed tube such as illustrated in Figs. 18 and 20.

When using the rubber tube stopper, with an integral rubber tube extending from the stopper, as shown in Figs. 11 and 14, the clamping sleeve, e. g. of annealed brass of suitable thickness, may be squeezed firmly with pliers that close with flat and parallel jaw surfaces and with a compound lever action to exert high pressure. The rubber tube should be squeezed down a short distance from the flat part of the stopper. The tube can then be cut off a short distance above the seal. This kind of clamp insures a vacuum-tight seal on the rubber exhaust tube.

The vacuum is maintained throughout the process and during the sealing operation. The vacuum is now turned off and air is admitted to the apparatus by means of the glass stopcock at the top of the main condenser in the apparatus of Fig. 1 or by withdrawing one of the manifold stoppers from a manifold outlet in the apparatus of either Fig. 1 or Fig. 5. Where glass exhaust tubes are used and are sealed with a flame the vacuum in the apparatus is not destroyed by the sealing of the tube. With rubber exhaust tubes the tube is sealed while the vacuum is maintained but the tube is not severed until the vacuum has been destroyed. Where rubber stoppers are used they may be covered with a suitable cement to further promote vacuum-tight sealing of the product.

If the volume of ice in the condenser leaves sufficient capacity for the next run, it is not necessary to empty the condensers, but one or more additional runs can be carried out provided the condenser capacity is adequate. If the condensers have insufficient capacity remaining for the next run they must be emptied. This requires melting of the ice in the condensers and the removal of the water from the condensers before the next run is begun. Melting of the ice can be accomplished by surrounding the condensers with warm water at 30 to 50° C. and by then siphoning out the condensate in the condenser when it has thawed, which can be accomplished by removal of the stopcock plug in the apparatus of Fig. 1 or by disconnecting one of the outlets of the main condenser in Fig. 5 and introducing a rubber siphon tube. The secondary condenser is easily disconnected to permit pouring out of any condensate. Once the condensate has thawed the condensers should not be packed with dry ice until they have been emptied. Otherwise the freezing of the condensate in the condensers would break or injure the condensers due to expansion of the water on freezing.

In apparatus of Fig. 1 it will be noted that the main condenser extends somewhat above the top of the surrounding container and that the inlet tube extends down to about the level of the top of the container while the outlet tube is at a higher level. In the metal apparatus of Fig. 4 the top of the main condenser is somewhat below the top of the surrounding container but the level of surrounding liquid at the outset is a little below the flat top of the main condenser so that the top of the main condenser is surrounded by an atmosphere of carbon dioxide. The arrangement in both cases is such as promotes rapid and effective condensation in the main condenser with a minimum of danger of plugging of the connecting pipes with the condensed ice.

In order to empty the condensers, the secondary condenser of Fig. 1 can be readily detached from the main condenser because of the rubber stopper connection; and the secondary condenser of Fig. 5 can similarly be disconnected by disconnecting the rubber tube which connects them.

After emptying the condensers, and before starting up the apparatus, all of the rubber connections on the condensers should be made before the condensers are chilled and they should not be disturbed while they are cooling, otherwise small pieces of ice condensed from the atmosphere tend to collect on the metal around the frozen rubber parts and cause leakage.

The laboratory apparatus described, and the method carried out with it, are adapted for use for a wide variety of purposes for the treatment and preservation of biological substances on a small scale such as for laboratory, research and control purposes.

The final containers, sealed while under a high vacuum, and without destroying the vacuum under which the dehydration was carried out, will contain the products preserved without danger of contamination from air or moisture, or from micro-organisms or other contaminants. It is possible to insure asepsis through the process so that the material finally sealed in the container in a dehydrated state has with certainty been protected from contamination after it was placed in the container and frozen.

The final product can be restored without destroying the vacuum, where a perforable rubber closure is used, as in Figs. 10, 11, 12, 14, 15 and 18 to 21. The rubber stoppers of Figs. 10, 11, 12, 15, 18, 19 and 20 have thin portions for the introduction of a hypodermic needle to admit liquid without destroying the vacuum. With the integral rubber tube stopper of Figs. 14 and 21 the rubber tube itself can be perforated with a hypodermic needle, etc., to admit water for restoration; and the rubber tubes of Fig. 19 can similarly be perforated instead of the short, separate, thin portion. The rubber stopper shown in Fig. 8 and also in various other figures has an opening extending part way through the stopper leaving a thin portion where the stopper can be readily perforated by a hypodermic needle.

The introduction of water or normal saline solution to restore the product, without destroying the vacuum, facilitates redissolving of the solid product since the product can be thoroughly wet with the water while the vacuum is maintained, and then, when atmospheric pressure is admitted, the pressure insures that the water is forced into all parts of the dry material and this thorough penetration of the water is not interfered with by contained gas; whereas, if the vacuum had been destroyed before the water was introduced, penetration would be retarded by the gas contained in the pores of the solid material.

The following table shows typical sizes of containers for use with the apparatus and process, with an indication of the approximate container volume, the maximum volume of serum or other material to be processed and to be contained in the container, the body length, diameter and wall thickness, and the length and diameter of the necks of the containers to adapt them for the rubber stopper closures by which an effective vacuum is to be maintained.

| Approximate container volume | Maximum serum volume | Body length | Body outside diameter | Wall thickness | Neck length | Neck inside diameter | |
|---|---|---|---|---|---|---|---|
| | | | | | | Large end | Small end |
| *Ml.* | *Ml.* | *Mm.* | *Mm.* | *Mm.* | *Mm.* | *Mm.* | *Mm.* |
| 100 | 50 | 185 | 35 | 2.0 | 15 | 15.2 | 13.7 |
| 50 | 25 | 110 | 28 | 1.5 | 15 | 15.2 | 13.7 |
| 25 | 13 | 80 | 22 | 1.5 | 15 | 15.2 | 13.7 |
| 12 | 6 | 60 | 20 | 1.2 | 15 | 15.2 | 13.7 |
| 6 | 3 | 45 | 15.5 | 1.0 | 15 | 15.2 | 13.7 |
| 2 | 1 | Sphere | 22 | 1.0 | 10 | 5.4 | 4.5 |
| 2 | 1 | 22 | 16 | 1.0 | 11 | 7.8 | 6.8 |

The size and shape of the containers are important in enabling the material to be frozen and the frozen material to be dehydrated with automatic self-regulation of temperature, with heating of the containers by the surrounding air and with cooling of the containers by the sublimation or evaporation of ice therefrom to maintain the product frozen and prevent melting during the carrying out of the process, and to enable a proper ratio to be obtained between the volume of the frozen material, the interior evaporating surface, and the exterior surface in contact with the walls of the container, as more fully described in my said companion applications.

Suitable rubber stoppers for use with the containers of the above table and for use in attaching the containers to the manifold and also for connecting the secondary condenser with the main condenser are shown in the following table:

| No. | | Body length | Neck diameter large end | Neck diameter small end | Diameter of exhaust hole |
|---|---|---|---|---|---|
| | | *Mm.* | *Mm.* | *Mm.* | *Mm.* |
| 1 | For carrying glass exhaust tube | 15.0 | 16.2 | 14.0 | 5.0 |
| 2 | Rubber exhaust tube-stopper | 16.0 | 16.8 | 15.1 | 6.5 |
| 3 | Small rubber exhaust tube-stopper | 10.0 | 9.0 | 7.5 | 4.0 |
| 4 | Secondary condenser attachment stopper | 15.0 | 16.2 | 14.0 | 8.0 |
| 5 | Manifold stopper | 15.0 | 16.2 | 14.0 | 5.0 |

The stopper No. 1 of the above table is illustrated in Figs. 8, 10 and 18. The rubber tube stopper of No. 2 is illustrated in Figs. 11, 16 and 19. The small rubber tube stopper No. 3 is shown in Figs. 14 and 21. Stopper No. 4 is stopper 7 of Fig. 1 for connecting the secondary condenser with the main condenser. The manifold stopper No. 5 is indicated at 36 in the drawings.

For high vacuum tightness, such as the maintenance of a high vacuum around 0.01 to 0.05 mm. during the process and when the final container is sealed, a great compression of the rubber of the rubber stopper is essential. This is obtained by tapering both the neck of the container and the stopper and by lubricating the stopper before it is inserted so that there is a maximum of compression on the entire length of the neck surface. A similar tight fit is obtained between the main and the secondary condenser and between the exhaust tubes and the manifold outlets to which the stoppers are attached.

In general the amount of material introduced into the individual containers will be around one-half or somewhat less of the volume of the container. The final dehydrated or lyophile product will have a volume approximately that of the original liquid or frozen material although in weight it will represent only a small fraction of the weight of the original liquid. The facility with which a small amount of a lyophilic product can be quickly restored to a liquid state with properties comparable with those of the original liquid material before treatment makes the present process and apparatus a valuable one for use on a small scale for experimental and laboratory purposes and the treating of a small number of multiple unit amounts of biological materials to preserve them for long periods of time such that they are available for clinical or other purposes.

The apparatus has proved well adapted for use in a number of laboratories for the preservation of sera of various kinds, such as a variety of normal and immune sera, viruses, enzymes, various proteins, bacterial cultures, human milk, etc.

In this application I claim the new apparatus herein described for use in the dehydration of biologicals and the like. The new process is claimed in my divisional application Serial No. 126,057, filed February 16, 1937.

I claim:

1. An apparatus for the dehydration of biological substances in a frozen state in small in-

dividual containers comprising a manifold having a number of upwardly and outwardly extending outlets, each adapted for receiving with a vacuum-tight fit a rubber stopper attached to the exhaust tube of a container of the frozen material, a main condenser directly connected to the manifold, a secondary condenser connected to the first condenser and in turn adapted to be connected to a vacuum pump, a container for containing both the main condenser and the secondary condenser with space therein for surrounding the condensers with a mixture of Dry Ice and organic solvent, the connection between the main condenser and secondary condenser being above the top of the container, and the inlet to the main condenser extending downwardly a short distance into the main condenser and terminating in a downward direction at a level lower than the outlet from the main condenser to the secondary condenser.

2. An apparatus for the dehydration of biological substances in a frozen state in individual containers under a high vacuum comprising a manifold having a number of upwardly and outwardly extending openings, each adapted to receive with a vacuum-tight fit the rubber stopper of the exhaust tube of an individual container, a number of individual containers for the frozen material each with an L-shaped exhaust tube, one end of which extends downwardly through the rubber stopper into the opening in the manifold, when the container is attached thereto, a condenser directly connected with the manifold, an insulated container surrounding the condenser and adapted to contain the mixture of Dry Ice and organic liquid therein around the condenser, said condenser having an inlet opening extending downwardly a short distance into the condenser and directing the vapors downwardly therefrom into the condenser, and said condenser also having an outlet arranged at a higher level leading to a vacuum producing means.

3. An apparatus such as set forth in claim 1 in which the main condenser and manifold are of glass and are made integral with each other, and in which the top of the main condenser is arranged above the top of the container, and the inlet tube to the main condenser extends down to a point about even with the top of the container surrounding the condenser.

4. An apparatus such as set forth in claim 1 in which the manifold and condensers are of metal, with the manifold directly connected to the top of the main condenser and having a discharge tube extending downwardly a short distance into the main condenser and in which the secondary condenser is of general U-shape and connected with the main condenser through a rubber tube connection.

5. An apparatus such as set forth in claim 1 in which the connection between the main condenser and secondary condenser is located above the top of the surrounding container and in which the inlet to the main condenser extends down into the main condenser and terminates at a location approximating the top of the surrounding container.

6. An apparatus such as set forth in claim 1 in which the secondary condenser has an inlet at the top and a central tube extending downwardly to near the bottom of the secondary condenser and in which both the inlet and outlet of the secondary condenser are arranged above the top of the surrounding container.

7. An apparatus such as set forth in claim 1 in which both the main and secondary condensers are made of glass and secured together through a rubber stopper connection with a vacuum-tight fit.

EARL W. FLOSDORF.

## FROM DEFENDANT'S EXHIBIT 1.

### Examiner's Statement.

[267] Div. 49—Rm. 124 NF:SP Paper No. 20

Department of Commerce
UNITED STATES PATENT OFFICE
Richmond, Va.

| | |
|---|---|
| In re application of:<br>Earl W. Flosdorf et al<br>Ser. No. 219858<br>Filed July 18, 1938<br>For: Biological Apparatus, Containers and Methods | Before the<br>Board of Appeals<br>On Appeal |

This is an appeal from the action of the Primary Examiner in finally rejecting Claims 1 to 5, 13, 14, 20, 22 to 25, 27, 28, 34 to 36 and 39 of the above identified application, Claims 6, 19, 21 and 29 to 33 having been cancelled, and Claims 7 to 12, 15 to 18, 26, 37, 38 and 40 having been allowed.

The appealed claims are as follows:*

* * * * * * *

[270] *Claim 28.* The method of treating and packaging biological material for preservation which comprises, filling at least one clinical dose into a receptacle to not more than 75% of its capacity, said receptacle being suitable as a final container adapted for protection, distribution and restoration for use of contents, dehydrating the bio-

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* The claims which were withdrawn at the beginning of the trial in the District Court have been omitted.

logical material by sublimation through the action of (1) a solid, porous chemical desiccant heat-regenerable without fusion and (2) vacuum until dry, while maintaining the material frozen by the autorefrigeration of the sublimation, and sealing said receptacle to produce a final package ready for storage or distribution.

* * * * * *

*Claim 35.* The method of treating and packaging biological material for preservation which comprises filling at least one clinical dose into a receptacle to not more than 75% of its capacity, said receptacle being suitable as a final container adapted for protection, distribution and restoration for use of the contents thereof, dehydrating the biological material by sublimation through the action of (1) a solid, porous chemical desiccant heat-regenerable without fusion and (2) vacuum until dry, and sealing said receptacle to produce a final package ready for storage or distribution.

* * * * * *

*Claim 39.* An apparatus providing means for treating biological material for preservation by dehydration under vacuum comprising means defining a chamber, drying means disposed within said chamber for removing water vapor from air within said chamber, a plurality of outlets suitable for use as container connectors communicating with said chamber, means controlling the communication of said outlets with said chamber and vacuum producing means communicating with the chamber for the removal of air therefrom.

## *Examiner's Statement.*

Serial No. 219858—5

[271] The references relied on are:

(1) References cited to show classification:

| | | | | |
|---|---|---|---|---|
| I. | Smith | 1,718,590 | June 25, 1929 | 128/272<br>(originally) |
| II. | Leetz | 2,079,514 | May 4, 1937 | 34/24<br>(now)<br>83/94 |
| III. | Slagel | 1,839,350 | Jan. 5, 1932 | 183/4 |
| | Paisley et al. | 2,100,610 | Nov. 30, 1937 | 183/49 |

(2) References cited against the claims:

| | | | |
|---|---|---|---|
| Hart et al | 653,555 | July 10, 1900 | 34/1 uxr |
| Hammond | 1,887,349 | Nov. 8, 1932 | 34/1 uxr |
| Morel | 864,978 | Sept. 3, 1907 | 34/5 |
| Flosdorf | 2,163,996 | June 27, 1939 | 34/76 |
| Elser | 1,970,956 | Aug. 21, 1934 | 34/Freezing (uxr) |
| Reichel | 2,066,302 | Dec. 29, 1936 | 34/Freezing (uxr) |
| Moore et al. | 2,083,732 | June 15, 1937 | 183/4 |
| Krause (Br.) | 12,642 | A. D. 1905 | 34/5 |

(2a) Reference cited for information:

| | | | |
|---|---|---|---|
| Follett | 137,835 | April 15, 1873 | 34/5 xr |

The British patent to Krause is cited under the provisions of ex parte Mevey, 1891 C. D. 115.

The application relates to a method and apparatus for the concentration, drying and preservation of biological substances such as sera, guinea pig complement, vaccines, breast milk, etc. The process includes such steps as degassing, self-freezing and dehydration by sublimation through the action of a regenerable chemical desiccant and vacuum.

The application further relates to a receptacle or package for clinical use for use as a container for individual doses or multiples thereof during dehydration, sealing, storing, distribution and restoration of the biological material.

The invention further relates to a filter or "air dryer and sterilizer" for admitting dry sterile air to the container just prior to the sealing thereof.

[272] Referring to Fig. 1, the apparatus consists of a vacuum-desiccant chamber 21 with one or more manifolds 58, 59 attached to the chamber, each manifold being provided with means for the attachment of a plurality of containers, and a vacuum producing means 22 which communicates with the chamber. The chamber is filled with a solid, porous chemical desiccant, which is heat-regenerable without fusion, such as anhydrous calcium sulfate, which is known by the trade name of "Drierite".

The manifolds consist of tubes which are provided with a plurality of outlets for the connection thereto of the containers. As illustrated in Fig. 3, the manifold 58 is provided with outlet tubes 86, in each of which a vacuum-tight valve 52 is provided. Tube 86 receives the rubber tubing 87 which holds the glass connector 51 from container 53 in place. Rubber tubing 88 connects the tube 51 to the neck 89 of the container 53. Trays 54 support the containers during the dehydration. The trays 54 may or may not be used to support freezing mixtures for the rapid chilling of the containers.

The different types of containers are illustrated in Figs. 18, 19, 20 and 22. When rubber stoppers are desirable, the type of container shown in Fig. 19 or Fig. 20 may be used. An all glass type of container is illustrated in Fig. 18. The containers may be made of ordinary glass.

The filter or "air dryer and sterilizer" is illustrated in Fig. 13 and consists of an ordinary syringe barrel 115 with a standard Luer needle 110 attached to it. The needle is used to pierce the rubber connectors 88 (Fig. 3) for the admittance of dry sterile air. In the bottom of the barrel

115 and occupying about ¼ of the space is placed a bacterial filter such as sterile cotton 116. 1/3 of the remaining space is filled with a regenerable chemical desiccant 117 such as "Drierite" and on top of this is placed another cotton plug 116 occupying about ½ of the remaining space. [273] Three methods of dehydration procedure are disclosed:

(1) Prefreezing—The containers are filled to approximately ½ of their capacity and attached to the manifold. The material is frozen and maintained at a temperature of approximately—10°C by spreading a salt-ice mixture over and around the containers on the tray 54. Mechanical refrigeration or DRY-ICE may be substituted for the salt-ice mixture. With the material in the frozen state and the containers 53 properly attached to the manifold, the evacuation is carried out to the limit of the capacity of the pump 22. After the desiccation is complete, the temperature is allowed to rise gradually to that of the room at such a rate that the temperature at any time is never much above that necessary to produce a small further differential in aqueous tension.

(2) Degassing Self Freezing—For certain materials containing protein &/or dissolved gas. After the filled containers have been connected to the manifold, the vacuum pump 22 is started, but instead of permitting the pump to operate at its full capacity, it is throttled down by means of valves 37 and 38 (Fig. 1) so as to keep the pressure at a point where frothing just begins to occur. As the gases are removed, the pressure may be further lowered. After the gases have been removed, valve 38 is closed and valve 37 fully opened. As soon as the pressure falls to the range of 2.5 to 1.0 mm. freezing of the material takes place and drying from the frozen state by sublimation proceeds. The vapors are adsorbed by the desiccant.

(3) Self-Freezing-Dehydration—This is similar to (2) above, the difference being that the degassing step is omitted.

After the material is completely dried, the container is sealed. When the material must be preserved in an aseptic condition, the procedure followed is to shut the individual manifold valves 52 (Figs. 3 and 11) and admit dry, sterile [274] air to an evacuated container 108 as shown in Fig. 11. After the dry sterile air has been admitted, the exhaust tube can be constricted at 111 to a diameter of about 1-2 mm. by means of a gas flame 112. As soon as constricting is finished, valve 52 is again opened and re-evacuation of container 108 proceeds. After a few minutes of evacuation, container 108 is ready for the sealing operation and is sealed, Fig. 12, by applying a gas flame 112 at section 114. The container is severed from the assembly and the finished product is as shown in Fig. 12*a*.

The sealing may be non-aseptic in which case the admission of sterile air is omitted.

For certain products, sealing in vacuo is unnecessary. The container is hermetically sealed to exclude moisture.

The apparatus disclosed may be used to regenerate the desiccant. When so used, valves 37 and 38 (Fig. 1) are closed and valve 60 opened to the air-heater-circulator 24. The cover 26 is removed and replaced by a loose-fitting disc 27, Fig. 2, for the purpose of permitting the air to escape slowly as it rises between the desiccant baskets 46 or the desiccant 44. The regenerating temperature required is between 150° to 250°C.

## Description of the References

### (1) References cited to show classification

I. *Smith* discloses and claims an ampule or package for clinical use.

II. *Leetz* discloses and claims a method of drying and disintegrating sea-ooze in which the sea-ooze is first subjected to a draft of air to effect drying, then frozen, then melted and again subjected to a draft of drying air.

III. *Paisley et al.* and *Slagel* disclose and claim filters which are somewhat similar in structure to applicant's filter or "air-dryer-sterilizer".

[275] (2) References cited against the claims:

*Hart et al.* disclose broadly the use of a solid chemical desiccant for removing water vapor from an air stream. They show a drying chamber 1, desiccant chamber 32, condenser 10, fan 16 and heater 19, all in series in a closed circuit. Hats containing solvent and moisture are placed in the drying chamber and air is recirculated through the said closed circuit. Quicklime, potassium carbonate or similar material is placed in the desiccant chamber to remove water from the circulating air stream. The solvent vapors are thereafter removed from the air stream by the condenser 10.

The patent to *Hammond* is for the compound anhydrous calcium sulfate ("Drierite"). He discloses that it is a drying or desiccating agent of the highest efficiency.

*Morel* discloses a process for obtaining dry gluten in which the wet gluten is placed in a bell i, the interior of which communicates through an outlet g with a desiccant chamber 6 containing sulfuric acid at 66°B. The desiccant chamber in turn communicates with a vacuum pump. The process is very similar to applicants' procedure, "Degassing-Self-Freezing". The gluten is placed in the bell on plates j and a low vacuum is produced in the interior thereof. The pressure is gradually decreased to about 1 mm. of Hg. Freezing of the water in the gluten is brought about by the action of the vacuum. The plates j are then heated electrically to a temperature of about 120°C. Under the combined action of the vacuum and heat, the water passes suddenly from a solid state into a state of vapor. As soon as a portion of the ice melts, the liquid is instantaneously evaporated by the action of the vacuum. The vapor thus formed is absorbed immediately by the sulfuric acid. The sudden evaporation and immediate absorption of the vapor causes the gluten to remain frozen until it is completely dehydrated.

[276] *Elser* discloses a process and apparatus for drying liquids and semi-solid materials containing proteins or other biological materials such as sera. Referring to Figs. 1 and 2, a series of containers A are connected by tubes a to a condensing chamber B which is connected by means of the tubes 1, 2 and 3 to the remainder of the drying apparatus illustrated in Fig. 1 A. The containers are maintained at a temperature slightly below 0°C (approximately —3°C) by being submerged in a suitable coolant contained in the tank 5. After the sera is frozen, air is withdrawn from the condensing chamber and evaporation by sublimation of the sera takes place. The evaporation of the liquids cooperates with the coolant to maintain the low temperature (lines 51 and 52, page 3). (This is partial auto-refrigeration).

One end of the chamber B is connected to a high vacuum pump C through a series of traps and driers as illustrated in Fig. 1 A. Vapor traps 7 and 12 are cooled, but before the withdrawn air reaches the pump it passes through one or more drying tubes 13 which are partly filled with a water vapor absorbing powder such as phosphorous pentoxide.

Upon completion of the drying operation, the containers are removed and corked (sealed).

Elser states that "this system neither depends upon nor contemplates chemical absorbents for performing the desiccation. Although such agents, as for example, phosphorous pentoxide have a powerful affinity for water, and a very low vapor pressure, nevertheless they (or perhaps, certain impurities thereof) are volatile to the extent of their vapor pressures, and may bring about changes in the serum such as to defeat the purpose of the invention", lines 74 to 83, page 1.

### *Examiner's Statement.*

*Reichel* discloses a method and apparatus for drying lyophilic sera and other biologically active substances. In [277] the carrying out of the process, the serum is placed in the container 1 and immersed in liquid air or other refrigerant which will bring about rapid freezing of the material. When the container has thus been charged, it is connected with the condenser 5 and a high vacuum applied. When the vacuum becomes sufficiently high, water vapor leaves the frozen charge without melting of the charge (auto-refrigeration) and is collected in the form of a layer of ice 10 on the walls of the condenser.

The material is maintained in a solid frozen state and at a low temperature until the ice has been sublimed therefrom, when the container and its charge gradually takes on the temperature of its surroundings. The high vacuum is continued until the substance attains a temperature substantially above 0°C, this having the advantage of promoting the removal of residual water in the product after the ice has been sublimed therefrom and giving a final product of improved stability, keeping properties and resistance to deterioration.

The products can be kept for prolonged periods of time by sealing in evacuated containers.

Reichel states "that the material is protected, during the removal of the water therefrom . . . from contact with or use of any of the known desiccating materials", lines 42 to 47, column 2, page 3.

*Flosdorf* discloses a method and apparatus for drying biologically active substances such as sera, protein substances, bacterial cultures, viruses, etc.

The apparatus consists of a manifold 1 having a plurality of outlets 2, which manifold communicates with a main condenser 3, an auxiliary condenser 4 and a vacuum pump through pipe 14.

In the operation of the apparatus, the material is introduced into small individual containers, frozen, and the containers are then connected to the exhaust tubes or manifold outlets 2. While in the frozen state, the material is subjected to vacuum dehydration with automatic self-regulation of temperature and this operation is continued until [278] the dehydration has been carried to the proper extent which is attained after the removal of the ice is completed and the product is thereafter warmed up to around room temperature and kept at that temperature while the vacuum is maintained to insure completion of the dehydration.

Upon completion of the dehydration, the containers are sealed while under a high vacuum, and without destroying the vacuum under which the dehydration was carried out. Two types of seals are illustrated in Figs. 18 and 19.

*Moore et al.* disclose the use of solid, porous adsorbents such as activated alumina, adsorbent gels and the like, to remove moisture from air and gases for various technical and industrial uses. The said adsorbents are heat regenerable without fusion.

*Krause* discloses a process for drying meat, poultry, eggs, fish, etc. The material is frozen and chloride of calcium or other hygroscopic agent may be provided for the purpose of absorbing the moisture. The chloride of calcium is placed in large cups on horizontal shelves, the cups being protected by wire netting upon which the frozen material to be dried is placed. As soon as the drying room is filled, it is sealed and as great a vacuum as possible is produced therein and maintained until the drying action is finished. The vapor generated during the drying by sublimation comes at once into contact with the chloride of calcium and is absorbed thereby.

*Follett,* cited for information, discloses that chloride of calcium may be regenerated with or without fusion. Lines 22 to 25, column 2, page 1, state that "chloride of calcium is easily restored by simply evaporating the moisture it contains after using it, and thoroughly drying it *or* fusing it by means of heat".

## APPLICATION OF THE REFERENCES

### (1) References cited to show classification.

Claims 1 to 3, 22 to 24 and 27 were rejected on the ground [279] of misjoinder of invention.

I. Claims 1 to 3, drawn to a package for clinical use, are thought to be more properly classified in Class 128 along with the patent to Smith.

II. Claims 4, 5, 7 to 18, 20, 25, to 28 and 34 to 40, drawn to a drying method and apparatus, are thought to be properly classified in Class 34. Although the patent to Leetz has been transferred from Class 34 to Class 83 (because of the combined disintegration and drying), the patents to Morel (34/5) and Flosdorf (34/76), above cited, show that these claims are properly classified in Class 34. These claims were elected for prosecution in response to the requirement of division.

III. Claims 22 to 24 and 27, drawn to an air drying filter, are thought to be more properly classified in Class 183 along with the patents to Slagel and Paisley et al.

The inventions are believed to be distinct and mutually independent. They have acquired a separate status in the arts as shown by the cited patents and are subject to separate and divergent fields of search.

### (2) References cited against the claims.

Claims 4, 5, 25, 28 and 35 were rejected as unpatentable over Flosdorf, Elser or Reichel in view of Hammond or Moore et al. It does not appear to involve invention to substitute a chamber containing a regenerable, solid, porous chemical desiccant for the condenser of Flosdorf, Elser or Reichel in view of the disclosure of Hammond or Moore. While the statements against using chemical desiccants by Elser and Reichel may be valid against certain volatile, poisonous desiccants, they cannot be valid arguments against the use of harmless, non-poisonous, non-volatile desiccants such as disclosed by Hammond or Moore. The apparatus, and method steps recited in these claims are merely conventional, except for the desiccant which use [280] is old and well known as shown by the reference Hammond or Moore.

Claims 4, 5, 25, 28 and 35 are also considered to be unpatentable over Flosdorf, Elser or Reichel in view of Krause. For the reasons stated in the preceding paragraph, and in view of the disclosure of Krause, it does not appear to involve invention to substitute a chamber containing calcium chloride for the condenser of Flosdorf, Elser or Reichel. Follett discloses that calcium chloride is regenerable with or without fusion. Furthermore, calcium chloride is obviously heat-regenerable without fusion under certain conditions of pressure and temperature and Claims 28 and 35 do not point out under what conditions of pressure and temperature the desiccant therein recited is heat-regenerable without fusion.

Claim 4 was further rejected as unpatentable over Morel in view of Hart et al. or Moore et al. To substitute the desiccants disclosed by Hart or Moore, or other solid desiccant such as calcium chloride for the sulfuric acid of Morel does not appear to involve invention. Follett teaches that calcium chloride is a regenerable desiccant. Applicants have merely substituted a solid, regenerable desiccant for the liquid, regenerable desiccant of Morel.

Claim 4 is also considered to be unpatentable over Morel in view of Krause. Krause discloses the use of calcium chloride as a desiccant in a combination which is similar to the combination recited in Claim 4 and disclosed by Morel. Therefore, it does not appear to involve invention to substitute calcium chloride for the sulfuric acid of Morel.

Claim 13 was rejected as unpatentable over Elser in view of Hammond or Moore as Claim 4. Note the tray 5 of Elser, Fig. 1.

Claim 13 is also considered to be unpatentable over Elser in view of Krause as Claim 4.

[281] Claims 14 and 20 were rejected on the ground of aggregation. There is no combination between the puncturing means and the rest of the apparatus any more than there is a combination between an automobile engine and a spark plug wrench. The puncturing means does not act on the material when the drying apparatus (desiccant chamber, desiccant, vacuum producing means) is acting thereon because valve 52 is closed. The puncturing means does not therefore modify the action of the elements of the drying apparatus, but operates independently to perform only its usual function.

Claim 34 was rejected as fully met by Elser.

Claim 36 was rejected as unpatentable over Elser or Flosdorf in view of Hammond or Moore as Claim 4. Merely to provide the container connectors a of Elser or 2 of Flosdorf with valves if desired, does not appear to involve invention.

Claim 36 is also considered to be unpatentable over Elser or Flosdorf in view of Krause as Claim 4, for the reasons stated in the preceding paragraph.

Claim 36 was further rejected as unpatentable over Morel in view of Hart or Moore as Claim 4. Merely to provide Morel with a plurality of outlets g and valves therefor does not appear to involve invention.

Claim 36 is also considered unpatentable over Morel in view of Krause as Claim 4, for the reasons stated in the preceding paragraph.

Claim 39 was rejected as unpatentable over Elser. Merely to provide Elser with a means controlling the communication between the tubes a and the air drying chamber 7, if not already so provided, (note the valve illustrated in the pipe 3, Fig. 1-A) does not appear to involve invention.

Respectfully submitted,

Examiner, Div. 49.

# [282] FROM DEFENDANT'S EXHIBIT 1.

## Decision of the Board of Appeals.

Appeal No. 42,770 MM

Hearing: June 24, 1942

IN THE UNITED STATES PATENT OFFICE

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BEFORE THE BOARD OF APPEALS

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*Ex parte* EARL W. FLOSDORF, CHARLES J. WESTIN and FRANCIS JOSEPH STOKES, JR.

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Application for patent filed July 18, 1938, Serial No. 219,858. Biological Apparatus, Containers and Methods.

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MESSRS. PENNIE, DAVIS, MARVIN & EDMONDS for applicants.

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This is an appeal from the decision of the examiner finally rejecting claims 1 to 5, 13, 14, 20, 22 to 25, 27, 28, 34 to 36 and 39.

The following claims are representative:

> 1. A package for a dry, very porous biological substance of superior stability in amounts suitable for clinical use, conveniently and easily restorable to original consistency by adding water and occupying approximately the same space in the package as the original liquid and adapted to contain the biological substance during filling, freezing, sealing, storing and use comprising, a sealed container of ordinary glass not having a particularly low co-efficient of thermal expansion.

4. An apparatus providing means for treating biological material for preservation by dehydrating through the action of a regenerable chemical desiccant and vacuum and for regenerating the desiccant comprising, a desiccant chamber, a solid, porous, regenerable chemical desiccant within the chamber, a plurality of outlets communicating with said chamber and for [283] use as container connectors and vacuum-producing means communicating with said chamber for the removal of air.

22. In an apparatus providing means for treating biological material for preservation by dehydrating through the action of a regenerable chemical desiccant and vacuum and for regenerating the desiccant, an air dryer and sterilizer comprising a hollow barrel, a supply of regenerable chemical desiccant located within said barrel, and a hollow needle connected to said barrel, there being an opening provided through said barrel and through said needle forming a continuous passage through said desiccant.

The references relied on are:

| | | |
|---|---|---|
| Hart et al | 653,555 | July 10, 1900 |
| Krause (Br.) | 12,642 | A.D. 1905 |
| Morel | 864,978 | Sept. 3, 1907 |
| Hammond | 1,887,349 | Nov. 8, 1932 |
| Elser | 1,970,956 | August 21, 1934 |
| Reichel | 2,066,302 | Dec. 29, 1936 |
| Moore et al. | 2,083,732 | June 15, 1937 |
| Flosdorf | 2,163,996 | June 27, 1939 |

The subject matter in issue has been described by the examiner as follows:

> "The application relates to a method and apparatus for the concentration, drying and preservation of biological substances such as sera, guinea pig complement, vaccines, breast milk, etc. The process includes such steps as degassing, self-freezing and dehydration by sub-limation through the action of a regenerable chemical desiccant and vacuum.

> The application further relates to a receptacle or package for clinical use for use as a container for individual doses or multiples thereof during dehydration, sealing, storing, distribution and restoration of the biological material.
>
> The invention further relates to a filter or 'air dryer and sterilizer' for admitting dry sterile air to the container just prior to the sealing thereof."

The examiner has required division on three lines.

I

> Claims 1, 2 and 3 directed to a clinical package.

II

> Claims 4, 5, 7 to 18, 20, 25 to 28 and 34 to 40 directed to a drying method and apparatus. It will be noted [284] that a number of the claims of this group stand allowed.

III

> Claims 22 to 24 and 27 directed to a needle device for introducing dry sterile air into the package.

This requirement for division appears to be sound. The three groups listed above cover separate and independent subjects matter of invention. It is obvious that the package defined in Group I could probably be produced by apparatus or processes different from that disclosed in this case. Apparently the package differs but little from that produced by the apparatus disclosed in the patent of Flosdorf noted above. It is also apparent that the needle device covered by Group III could be used anywhere for introducing dry sterile air into a package. The three devices are separately classified and searched in this Office. We believe that the requirement for division should be sustained.

The rejection of claims 4, 5, 13, 14, 20, 35, 26 and 39 of Group II on the prior art is fully justified. The patent to

Flosdorf appears to be the most pertinent. In general, this patent discloses a manifold to which packages may be connected and absorbing material and a vacuum pump. Appellants lay considerable stress on the fact that in their device a desiccant is used which can be readily regenerated. Appellants propose to use calcium sulfate as the hygroscopic material. This material is quite porous and when heat is applied to regenerate the material by driving off [285] the absorbed water, the porosity of the absorbent is not destroyed. This is not true of calcium chloride which is most generally used as a hygroscopic agent. However, the specific hygroscopic agent used by appellants is shown to be old in the patent to Hammond cited above. The mere substitution of calcium sulfate for the phosphorous pentoxide disclosed in the patent to Flosdorf does not appear to amount to invention.

Claims 14 and 20 have been rejected as covering aggregations of unrelated apparatus. We agree with the examiner that there is no combination in a patentable sense between the means for freezing and evaporating water from a material by suction and adsorption, and the needle device disclosed in Fig. 13. These two pieces of apparatus must necessarily act in sequence. The exhausting operation producing a dried material is completed before the needle device is employed. The first piece of apparatus produces a high vacuum in the package and the second destroys it. This ground of rejection should be affirmed.

The wording of claims 22, 23, 24 is somewhat peculiar. If the first four lines of each of these claims are intended to make the exhausting apparatus a part of the combination, then these claims are also rejectable on the ground of aggregation.

The requirement for division between Groups I, II and III as outlined above is affirmed.

The rejection of claims 4, 5, 13, 14 to 20, 22, 23, 24, 27, [286] 35, 36 and 39 on prior art and for aggregation is also affirmed. The process defined in claims 25 and 34 does not appear to be met by the art cited and these two claims may be allowed.

| | |
|---|---|
| W. L. Redrow,<br>Examiner-in-Chief<br>J. W. Clift,<br>Examiner-in-Chief<br>C. H. Shaffer,<br>Examiner-in-Chief | Board of Appeals. |

Messrs. Pennie, Davis, Marvin & Edmonds,
247 Park Ave.,
New York, N. Y.

July 16, 1942

## [287] FROM DEFENDANT'S EXHIBIT 1.

### Decision of the Board of Appeals on Reconsideration.

Appeal No. 42,770 MM

IN THE UNITED STATES PATENT OFFICE

BEFORE THE BOARD OF APPEALS

*Ex parte* EARL W. FLOSDORF, CHARLES J. WESTIN and FRANCIS JOSEPH STOKES, JR.

Application for patent filed July 18, 1938, Serial No. 219,858. Biological Apparatus, Containers and Methods.

MESSRS. PENNIE, DAVIS, MARVIN & EDMONDS for applicants.

ON PETITION FOR RECONSIDERATION

Reconsideration has been requested in view of certain language used in our decision in connection with the Flosdorf patent. It must be conceded that our treatment of this reference is not as full and complete as it should have been.

This patent discloses small laboratory type of apparatus for carrying out substantially the same process as contemplated here, that is a product containing water is first frozen by evaporating the water content and then dehydrated by means of a high vacuum without permitting the product to thaw. It must be presumed that the frozen product is maintained at a temperature just below the freezing point of water. It is connected by tubing with the condensers sunk in a bath of solid $CO_2$ and an organic [288] liquid. By reason of the difference in temperatures the ice in the product will sublime and be collected and condensed in the condensers.

It is well known that water in passing from the solid or liquid to the vapor phase expands enormously and therefore it is common to use an absorbent to aid the vacuum pump or the condensers. Flosdorf apparently appreciated the fact that an absorbent such as $P_2O_5$ could be used, but preferred to rely on condensers alone. It appears from other references cited that phosphorous pentoxide is objectionable for this type of work, but the Follett patent shows that appellants' specific absorbent is old.

On page 3, second paragraph of our former decision, claim 26 was erroneously listed as one of the claims under rejection. Claim 26 stands allowed. Claim 28 was meant instead.

The petition is granted to the extent indicated, but for reasons now of record our former decision remains unchanged.

W. L. Redrow,
Examiner-in-Chief

J. W. Clift,
Examiner-in-Chief

C. H. Shaffer,
Examiner-in-Chief

Board
of
Appeals.

Messrs. Pennie, Davis, Marvin & Edmonds,
247 Park Ave.,
New York, N. Y.

Sept. 26, 1942

UNITED STATES
COURT OF APPEALS FOR THE
DISTRICT OF COLUMBIA

FILED JUN 17 1944

Joseph W. Stewart

CLERK

## BRIEF FOR APPELLEE

# United States Court of Appeals

DISTRICT OF COLUMBIA

## APPEAL NO. 8715

F. J. STOKES MACHINE COMPANY, APPELLANT
*v.*
CONWAY P. COE, COMMISSIONER OF PATENTS, APPELLEE

*APPEAL FROM THE JUDGMENT OF THE DISTRICT COURT OF THE UNITED STATES FOR THE DISTRICT OF COLUMBIA*

**W. W. COCHRAN,**
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# United States Court of Appeals

DISTRICT OF COLUMBIA

## APPEAL NO. 8715

F. J. STOKES MACHINE COMPANY, APPELLANT
*v.*
CONWAY P. COE, COMMISSIONER OF PATENTS, APPELLEE

*APPEAL FROM THE JUDGMENT OF THE DISTRICT COURT OF THE UNITED STATES FOR THE DISTRICT OF COLUMBIA*

## BRIEF FOR THE COMMISSIONER OF PATENTS

### INTRODUCTORY STATEMENT

This is an appeal from the judgment (16)[1] of the District Court of the United States for the District of Columbia dismissing the complaint (1) brought by F. J. Stokes Machine Company in which it was sought to have the Court adjudge that the plaintiff is entitled to receive a patent on application Ser. No. 219,858, filed on July 18, 1938, by its assignors Earl W. Flosdorf, Charles J. Westin and Francis Joseph Stokes, Jr., containing claims 28 and 35 of said application.

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[1] The numbers in parentheses throughout this brief refer to pages of appellant's appendix.

## STATEMENT OF THE CASE

The Flosdorf application, involved herein, relates to a process of, and apparatus for, treating and packaging biological material, such as sera, guinea pig complement, vacines, etc. In carrying out the process the biological material is placed in a receptacle, which is suitable for the protection of the contents and restoration for use by adding water thereto. This receptacle is connected to a vacuum apparatus containing a regenerable chemical desiccant, preferably a form of calcium sulphate, known to the trade as "Drierite," then freezing the biological material into a solid and rapidly reducing the pressure so that water vapor is given off from the frozen material by sublimation and is absorbed by the desiccant until the material is dry, closing the connection between the apparatus and the receptacle, admitting sterile air to the latter and sealing off the tube. The material is then in a dry fluffy condition and when it is desired to use it, water is admitted to the receptacle, and is absorbed by the material.

A number of claims were presented in the application.

The examiner cited among others, the following patents:

Flosdorf, No. 2,113,996;
Elser, No. 1,970,956;
Reichel, No. 2,066,302;
Moore et al., No. 2,083,732; and
Hammond, No. 1,887,349.

The Flosdorf patent discloses a process and apparatus for treating biological materials, which are of the same type as that shown in the application here involved, but differing therefrom in that instead of the water vapor being absorbed by a desiccant, it is condensed to ice in a condenser and removed therefrom by sublimation.

The patent to Elser No. 1,970,596 and Reichel No. 2,066,302 each shows a process for desiccation of biological materials in which, as in the Flosdorf patent, a condenser is employed for effecting the removal of the water vapor.

The Moore et al. patent shows the use of solid, porous desiccants, such as activated alumina, absorbent gels and the like, for removing moisture from air and gases for various technical and industrial uses.

The Hammond patent No. 1,887,340 describes a desiccating agent, which may be either sulphate of lime or calcium sulphate, the lafter being the material which is designated in the application in suit as "Drierite." This patent states that the calcium sulphate is a desiccating material of the highest efficiency.

The examiner (See Examiner's Statement App. 137) rejected certain of the claims presented but allowed 11 method claims and 6 apparatus claims. The Board of Appeals, while in general affirming the action of the examiner, allowed two additional method claims Nos. 25 and 34, which are narrower than the claims appealed.

Claims 28 and 35 are very broad and differ from the Flosdorf patent or the Elser and Reichel patents only in the limitation "dehydrating the biological material by sublimation through the action of (1) a solid porous chemical desiccant heat-regenerable without fusion and (2) until dry."

Claim 28 reads as follows:

> 28. The method of treating and packaging biological material for preservation which comprises, filling at least one clinical dose into a receptacle to not more than 75% of its capacity, said receptacle being suitable as a final container adapted for protection, distribution and restoration for use of contents, dehydrating the biological material by sublimation through the action of (1) a solid porous chemical desiccant heat-regenerable without fusion and (2) vacuum until dry, while maintaining the material frozen by the auto-refrigeration of the sublimation, and sealing said receptacle to produce a final package ready for storage or distribution.

Claim 35 is slightly broader than claim 28 since it omits the limitation "while maintaining the material frozen by the auto-regeneration of the sublimation."

The examiner based his rejection on the ground that, desiccants being known including the desiccant of the Hammond patent, and it being known as shown in the Moore et al. patent, that desiccants could be used for removing moisture from air and gases, it was not invention to use such a desiccant in the apparatus of the Flosdorf patent, in lieu of the condenser used therein. The Board of Appeals in its decision (150) took the same view.

Finding 8 of the Findings of Fact made by the District Court (15) reads as follows:

8. It was not invention to use a chamber containing a desiccating agent, such as disclosed in the patent to Hammond and in the patent to Moore et al., in lieu of the condenser of the Flosdorf patent or that of the Elser or Reichel patents.

## SUMMARY OF ARGUMENT

1. The burden in an action brought under the provisions of Sec. 4915 R. S. (U. S. C., title 35, sec. 63) is not upon the defendant to prove that the conclusion reached by the Board of Appeals of the Patent Office is correct.

2. It was not invention to employ in the process disclosed in any one of the patents to Flosdorf, Elser, and Reichel a desiccant in lieu of a condenser.

## ARGUMENT

Claims 28 and 35 of the Flosdorf et al. application are extremely broad. No details of the process or the conditions under which the process is carried out are included in these claims. They are broadly for a process of treating biological material and preserving it, in which a desiccant is used as the means for removing the water vapor sublimed from the biological material.

The tribunals of the Patent Office had done their duty when they studied the relevant prior art and reached a conclusion as to the patentability of the claims presented. If it was then thought the applicants were entitled to broader claims, as well as the narrower ones which had been allowed, the burden was on the plaintiff to establish that they were so entitled. As this Court has repeatedly said, the burden on the plaintiff is a "heavy one."

Again, this Court has said the presumption of correctness of the administrative ruling applies just as much to an adverse ruling as it does to a favorable ruling which results in the issuance of a patent (See *Magnaflux Corp.* v. *Coe,* — U. S. App. D. C. —, — F. (2d) —, 60 P. Q. 3.

Again, ever since the decision in *Abbott* v. *Coe,* 71 App. D. C. 195, 109 F. (2d) 449, 1940 C. D. 13, this Court has held that when the District Court makes a finding to the same effect as that of the Patent Office, the presumption of correctness of the administrative ruling is all the greater.

If appellant means what he says as to the defendant's having to prove the correctness of the administrative ruling, it would follow that unless and until the defendant brought forward in the District Court further evidence than the prior art cited, it would be the duty of the Court to find that plaintiff is entitled to a patent containing the claims which had been refused him by the Patent Office, a conclusion which is surely a *reductio ad absurdum.*

The art clearly shows the treatment of these biologicals to abstract therefrom all the water therein, so as to leave a dry material and enclose it in a sterile condition.

The applicants claim to have discovered that this can be done by the use, in an otherwise old process, of a well-known desiccant for absorbing the water vapor.

There is nothing in the application to show that each and every desiccant can be used for that purpose nor even any desiccant other than the material described in the Hammond patent and known in the trade as "Drierite."

The statement made in Mr. Flosdorf's testimony, and very much stressed in appellant's brief, is that it was believed by those skilled in the art that there was a likelihood that some minute particles of the desiccant might get in the biological material, which was being treated. He quoted (Tr. page 22) from both the Reichel and the Elser patents.

It is to be noted that neither of these patents makes any reference to the desiccant of the Hammond patent which the applicants use.

It is submitted that the art cited would clearly, as held by the Patent Office tribunals, suggest the use of that desiccant.

If certain desiccants, such as phosphorous pentoxide, have such characteristics as to make it dangerous to use them in lieu of a condenser in such processes as that of the Flosdorf patent, it does not follow that that would be true of "Drierite."

Certainly if one was apprehensive that "Drierite" might have such a property, it would not have been necessary to do any thing more than to test it.

It may be noted that Mr. Flosdorf in his testimony gave no explanation as to how he happened to use "Drierite" in lieu of a condenser. Nor does he say that he was of the opinion that it would have been dangerous to use "Drierite" as a means of removing water from the biologicals.

Nor is Mr. Hammond's statement to Mr. Flosdorf (App. 30) that he did not know that the desiccant of his patent could be used in any such process as that of Flosdorf, of any weight in determining the question involved in this case.

It does not appear that Hammond knew anything about the art in which Flosdorf was working or that he had any basis on which he could have formed any conclusion as to the probable effect of using "Drierite" in such a process.

Appellant relies heavily on two decisions of this Court in support of the argument for the patentability of the claims in suit, to wit: *Gasoline Products Company* v. *Coe,* 66 App. D. C. 333, 87 F (2d) 550, 1937 C. D. 39 and *Levin* v. *Coe,* 76 U. S. App. D. C. 347, 132 F. (2d) 589, 1942 C. D. 114.

It is submitted, however, that the facts of those two cases are so different from those of the present case and the grounds given for the Court's conclusion, so inapplicable to the present case, that these decisions would in nowise constitute a precedent for reversing the judgment of the District Court in this case.

In the *Gasoline Products* case, this Court held that claims for an apparatus for cracking petroleum oils, in which the cracking coils were formed of a chromium steel, were patentable. However, the reason for so holding given by the Court, speaking through Justice Stephens, was that the applicant was the first to discover that the cause of the corrosion in the coils was the sulphur or sulphur compounds in the oils, and the prior art did not teach that a chromium alloy was resistant to such corrosion.

Obviously no comparable situation is presented here.

In the other case, *Levin* v. *Coe,* this Court, again speaking through Justice Stephens, said that the generalization of *Paramount Corp.* v. *American Tri-Ergon Corp.,* 294 U. S. 464, 1935 C. D. 777, to the effect that the application of an old process to a new and closely analogous subject matter, was not "apt in the present case," since "appellant's process and resultant product are not old" and appellant accomplished "the satisfaction of an old and recognized want."

Those statements are not applicable to the present case. There is nothing here to establish that the product of the Flosdorf et al. application here involved was different in kind from the product of the Flosdorf prior patent.

Here the art clearly taught that water vapor could be removed from air and gases and the characteristics of the product of the Hammond patent were such as to indicate its use in such processes as that of the Flosdorf patent. If it had been thought that certain desiccants were not usable in those processes it did not follow that all desiccants were unusable and only a test would have been necessary to establish that "Drierite" was usable.

Attention is called to the recent decision (May 29, 1944) of the Supreme Court of the United States in *Universal Oil Products Co.* v. *Globe Oil & Refining Co.,* — U. S. —, in which the Court said:

> "Retrospective simplicity is often a misleading test of invention where it appears that the patentee's conception in fact solved a recognized problem that had baffled the contemporary art; but in this case Egloff advanced his improvement shortly after Dubbs disclosed the underlying process and before Dubbs' system had had wide commercial use; hence contemporary workers had no occasion to deal with whatever engineering problems might have been involved. We have, therefore, a conception which is on its face too obvious to constitute patentable invention, and which was advanced shortly after any need of it arose. We think the district court was right in finding the Egloff patent invalid."

It is submitted that the above quotation is clearly applicable to the facts of the present case.

## CONCLUSION

It is submitted that finding No. 8 of the District Court is not incompatible with the evidence and, on the contrary, is supported by the record. It is further submitted that the Conclusion of Law of the District Court is correct and the Judgment of that Court should be affirmed.

Respectfully submitted.

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JUNE 1944.

U. S. GOVERNMENT PRINTING OFFICE: 1944